Psychophysical properties and neural correlates of superficial and deep tissue pain

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Heather Cameron

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Contents

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BOLD	blood oxygen level dependent
DLPFC	Dorsolateral prefrontal cortex
VLPFC	Ventrolateral prefrontal cortex
OFC	Orbito-frontal cortex
PFC	Prefrontal cortex
ACC	Anterior cingulate cortex
PCC	Posterior cingulate cortex
pACC	perigenual cingulate cortex
aMCC	anterior mid cingulate cortex
pMCC	posterior mid cingulate cortex
CMA	cingulate motor area
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
MI	Primary motor cortex
SMA	Supplementary motor area
AI	Anterior insula cortex
rAI	Rostral anterior insula cortex
cAI	Caudal anterior insula cortex
PI	Posterior Insula cortex
VAS	Visual analogue scale
SF-MCG	Short Form McGill Pain Questionnaire
AUC	Area under the curve
ID	intradermal
SC	subcutaneous
MS	muscle

Abstract

The projects described in this thesis investigate the psychophysical properties and neural correlates of cutaneous and muscle pain in healthy volunteers and in a small patient group.

A total of four experiments are reported; two in healthy volunteers and two in patients with lateral epicondylitis.

The first experiment investigated psychophysical differences between intradermal, subcutaneous and intramuscular pain induced by hypertonic saline injections. The pain descriptors used by the subjects were different between the three injections. The profile of pain, including the intensity and time course and response to each single injection, was similar between intradermal and intramuscular injections whereas results were inconsistent with subcutaneous injections.

The second study, employing the methods developed in the first, used functional magnetic resonance imaging (fMRI) of the brain to undertake a comparison of the neural activation patterns associated with intradermal and intramuscular pain.

The results reveal commonalities but also distinct differences between the two tissue types, including more prominent activations following intramuscular injections in some brain areas including prefrontal cortex and caudate nucleus. In addition, peak activation following intramuscular injection was observed to be more rostral in a key component of the pain circuitry, anterior insula.

The third study compared brain activations in a clinical pain population with results suggesting that pain processing in this population involves ventrobasal structures (amygdala and hippocampus) which was not seen in healthy subjects in study 2. Activation in secondary somatosensory cortex appeared more posterior than in healthy subjects.

Innocuous and painful compression of the painful area in two subjects also revealed unique brain activations.

Taken together the studies reveal differences in both perceptual qualities and neural correlates of cutaneous and muscle pain; both in healthy volunteers and in clinical pain patients.

Chapter one: Introduction to the thesis

This first chapter serves to provide an introduction to the thesis and the topics covered therein.

It begins with a background to the topics presented; providing first a historical aspect of and then generic overview of pain; aiming to set the context for the investigations later presented.

The chapter concludes with a brief overview of the individual chapters contained within the rest of the thesis.

1.1 Historical perspective on pain

Historically the overriding theory of pain was the specificity theory proposed by Rene Descartes in the 17th century. Descartes argued that the human body worked akin to a machine thereby allowing its study using methods of physics (Melzack 1999). Although Descartes argued that humans did have a soul, it was separate from the body; the latter being a machine, rather like an animal's body. Pain he argued was therefore the result of a simple one way, un-modifiable system that occurred when a peripheral external stimulus sent a signal to the 'pain pathway', this signal being transmitted along the pathway to a single 'pain centre' within the brain.

This specificity theory dominated not just scientific investigation but also medical therapeutic practice for some time, persisting well into the 21st century.

Melzack and Wall (1970) in a review of pain related investigations and experiments conducted in the first half of the 20th century note that these predominately focused on the search for specific pain fibres, pathways and a pain centre in the brain. Thus the concept of pain as a specific straight through sensory projection system was accepted.

Several theories of pain however did follow, the major opponent to the specificity theory being pattern theories which although described as vague and inaccurate are considered to have 'set the stage' for the emergence of the gate control theory proposed by Melzack and Wall (1965).

However none of these successive theories considered an explicit role for the brain in pain other than as being a passive recipient for incoming information. Although they did signal a shift of focus in the direction from the periphery to the central nervous system albeit at the level of the spinal cord.

Melzack and Wall's proposal of the gate control theory of pain was the first to truly recognise that not only is the awareness of pain not directly linked to the degree of noxious stimulus presented i.e. that the signal could in some way be modulated but that this modulation may be driven by higher cognitive centres.

They proposed that transmission of nerve impulses from afferent fibres in the periphery to spinal cord transmission (T) cells is subject to modulation by a spinal gating mechanism within the dorsal horn. Further this spinal gating would be affected by activity in large and small diameter fibres; large fibre activity having an inhibitory effect on transmission i.e. would 'close' the gate whilst activity in the small fibres would have a facilitatory influence or 'open' the gate. The theory contended that this spinal gating mechanism is influenced by nerve impulses that descend from the brain via a specialised system of large diameter fibres that activate selective cognitive processes that influence, via descending fibres the properties of the spinal gating mechanism.

At the time of the proposal the gate control theory caused much debate and was seen as the catalyst to a great deal of research seeking to support or disprove the theory. Although the concept has been modified over the proceeding 30 years many principles of the gate control theory hold true today (Gifford 1998) and the concept is included in every major textbook on pain.

1.2 Definition of pain

Pain has been defined by the International Association for The Study of Pain (IASP, 1979) as 'an unpleasant sensory and emotional experience associated with actual tissue damage or expressed in such terms' i.e. pain is ultimately a perception; a perception determined by a number of factors, both internal and external to the person experiencing the pain, such factors may include previous experience / exposure to pain, the environment in which it occurs and the potential impact the pain may have on an individual's life.

The term pain should be differentiated from nociception; the latter refers to the mechanisms by which the nervous system handles noxious or unpleasant

information whereas the former refers to the actual physical and emotional sensation that an individual experiences.

Pain, both experimental and clinical, in common with any form of somatic sensation, is now considered to be the integration of sensory, cognitive and affective components (Melzack & Casey 1968); these three components accounting for the multidimensional complexity of pain. Sensory attributes include location, magnitude and spatiotemporal properties; affective properties include the hedonic aspect – i.e. the degree of unpleasantness of the sensation and a motivational aspect – producing the motivation to limit the experience of the noxious stimulus (Sewards et al., 2002). It has been argued that the hedonic and sensory aspects of somatic sensation are represented and processed in parallel but distinct pathways throughout the nervous system. Cognitive aspects of pain include the interpretation and meaning of the pain to the individual and again are subject to many factors including past experience and environmental situation (Sewards et al., 2002).

1.2.1 Taxonomy of pain

The taxonomy of pain over the years has been the subject of much debate (Merskey and Bogduk, 1994). Researchers and clinicians alike have sought to define pain syndromes both for academic and clinical progression. Pain may be classified as 'experimental pain' or 'clinical pain'; both of which however are generic and cover a broad spectrum of both normal physiology and pathophysiology. Experimental pain involves stimulation of nociceptors through the introduction of a noxious stimulus; stimuli may be chemical, thermal, electrical or ischaemic (Arendt Nielsen et al., 2001). Typically both the stimulus and the response will be relatively short lasting, causing little or no tissue damage and the effects of which are totally reversible. Experimental pain investigations are frequently undertaken in animal studies or healthy volunteers although increasingly these are also being applied in patient populations.

The term 'clinical pain' refers to a pain condition arising from injury or disease; typically where actual tissue damage has occurred. Clinical pain is frequently

further categorised based on temporal characteristics into acute or chronic pain and / or employing a tissue based classification into neuropathic, nociceptive or visceral pain (Merskey and Bogduk, 1994). The term 'acute' is typically employed in the early stages of injury e.g. post fracture or surgical operation, whilst 'chronic' pain has been defined as 'pain which persists past the normal time of healing' (Bonica 1953).

Neuropathic pain is defined as 'pain initiated or caused by a primary lesion or dysfunction in the nervous system' (Merskey and Bogduk, 1994). Further sub - classifications of neuropathic pain being peripheral neuropathic or central neuropathic pain; this relates to whether the symptoms originate from or are mediated predominantly by the peripheral or the central nervous system. Visceral pain is that which arises from the viscera whilst nociceptive pain typically arises from the musculoskeletal system (Merskey and Bogduk, 1994).

1.3 Generic mechanisms of pain

The development of the gate control theory developed by Melzack and Wall in the late sixties led to an upsurge in pain research and a greater understanding of the complexity of pain perception. In the periphery, the initial noxious stimulus (mechanical, thermal or chemical) activates primary sensory nerve cells with receptive properties, which are tuned to noxious stimuli (Julius and Basbaum 2001) but only if these stimuli are of sufficient intensity to cause actual or potential damage to tissue (Woolf and Ma 2007). These specialised nerve cells, nociceptors, rapidly receive, transduce and integrate noxious information (Hucho and Levine 2007), allowing the organism to take defensive or avoidant action. Aδ nociceptors, which are thinly myelinated and of medium diameter, belong to two main classes – type I are polymodal, responding to heat, mechanical and chemical stimuli (Meyer et al 2006) while type II fibres are less responsive to mechanical stimuli, respond more slowly to heat (with a slower conduction velocity than Type I fibres) yet are credited with the first pain sensation from a noxious heat stimulus. C-fibre nociceptors which are unmyelinated, polymodal and of small diameter, respond to noxious chemical, thermal and mechanical stimuli (Julius and Basbaum 2001). Aδ fibres, because of their larger size and more rapid

transduction (with a conduction velocity of 6 -25 ms^{-1}), are thought to be responsible for the immediate "first" sharp pain sensation, whilst the smaller C-fibres, firing more slowly (at a conduction velocity of less than 1.0 ms^{-1}) are the candidates for the transmission of the slower dull, more disseminated "second" pain sensation (Julius and Basbaum 2001). The first pain is perceived within 0.84 seconds whereas the second pain evolves over the next 2.1 seconds (Handwerker and Kobal 1993).

Nociceptive activity is modulated by mediators in the extracellular tissue fluid (Hucho and Levine 2007). When the peripheral terminals of C-fibres are stimulated enough to fire, they cause an immediate rise in the concentration of glutamate in the injured tissue which reaches a peak within a 2 hour period and transiently re-activates nociceptive nerve fibres (Ro at al., 2005). In addition to the release of glutamate there is a range of chemicals that act on the peripheral terminals of C-fibres, sensitising them and causing them to fire in response to innocuous, low-intensity stimuli, so that the pain sensation continues (Woolf and Ma 2007). These chemicals have been termed the "inflammatory soup"; these surround the area of tissue damage, and include cytokines, amines, prostanoids, growth factors, kinins and chemokinines. Nociceptive neurons, in common with all sensory neurons are capable of long-term plastic change in response to this chemical sensitisation, contributing to the experience of persistent pain (Hucho and Levine 2007).

Nociceptive afferent fibres terminate predominantly in the dorsal horn of the spinal cord and synapse with projection neurons located in lamina I and interneurons in lamina I and lamina II (Graham et al., 2007). A subset of these neurons, wide dynamic range neurons, responds to stimuli that are both noxious and non-noxious (Basbaum and Jessell, 2000).

Nociceptive signals then project to the brain via five key ascending pathways: spinothalamic, spinomesencephalic, cervicothalamic and the spinohypothalamic (Basbaum and Jessell, 2000). The spinothalamic tract is central to pain processing; lamina V dorsal horn neurons predominantly project Aδ and C nociceptor input directly to lateral thalamic nuclei via the contralateral

spinothalamic tract, whilst Lamina I-II dorsal horn neurons of the spinomesencephalic and spinoreticular tracts mainly relay C nociceptor input to parabrachial, midbrain, and medial thalamic nuclei (Hunt and Mantyh, 2001).

The thalamus is the main relay location for the passage of nociceptive signals to the cortex not only for the relaying of ascending signals, but also for the relaying of descending, pain modulating signals (Weiss et al., 2005)

Julius and Basbaum (2001) acknowledge that most of the work on primary nociceptive afferents has been done using a model of skin pain, as these are most accessible. However, nociceptors are spread widely throughout the body, innervating the skin, muscle, joints and the viscera and in each area of the body are specialised to respond to different stimuli, for example to light touch over the cornea, or to any stimulus to the teeth. Visceral silent nociceptors have a firing threshold that is dramatically reduced by inflammation and chemicals. (Basbaum and Jessell, 2000).

1.3.1 Modulation of pain

The descending pain modulatory system comprises a cerebral, spinal and peripheral network, such that pain may be either reduced or enhanced (Fields et al, 2006). Pain modulation is mediated in the main by endogenous opioid peptides that are ubiquitous in the pain matrix but particularly in the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM). Endogenous opioid release, and subsequent pain relief, is dependent upon behavioural triggers, sensory stimuli and the mediating action of neurotransmitters such as GABA and glutamate (Fields et al., 2006).

In addition, Yoshimura and Furue (2006) have discussed the analgesic effects of noradrenaline and serotonin release by the membrane hyperpolarisation, reducing the excitatory effect of nociceptive fibres, and stimulating the release the inhibitory transmitters, GABA and glycine.

1.4 Why do we feel pain?

Wall (1979) describes the sensation of acute pain as being the 'conscious signal of a physical threat'; as such pain may serve as a warning, a major purpose of which, in addition to producing the emotional reaction of fear and or anger, is to provide the motivation that may bring about an appropriate behavioural response facilitating both survival and healing. Depending upon the type of stimulus, the tissue involved, environmental factors and previous experience behavioural response may vary.

The initial response therefore of acute pain is purposeful; bringing about behaviours that remove the body from danger thereby reducing further damage and promoting healing. Behaviours can also bring about assistance – e.g. a child's cry or a back pain sufferer's grimace. When pain persists however the ensuing behaviour often no longer serves a purpose, indeed the opposite is frequently true. Protective or avoidant behaviours associated with chronic pain may contribute to the persistence of pain and its resistance to therapeutic interventions.

When faced with an actual or perceived physical insult a number of physiological responses occur, of which the perception of pain may or may not be one. Issues of bodily survival take precedent over the perception of pain and its associated illness behaviour (Gray 1987) as seen for example in the battlefield situation where injured American service personnel did not report pain in the immediate post trauma time period (Beecher 1946).

Indeed the suppression of pain by the CNS may be a survival strategy to prevent pain impinging on our consciousness (Fields & Basbaum 2006). Conversely pain experienced may far outweigh actual or potential tissue injury with respect to the stimulus.

Hence it is now well established that the reported experience in terms of severity and unpleasantness does not always correlate with the degree of noxious input / stimulation and / or tissue damage (Wall 2000). Nociceptors may fire with very little reported pain and conversely severe pain may be reported in the absence of

excessive noxious stimulus. Pain experienced and / or reported by an individual is frequently disproportionate to the magnitude of the actual nociceptive stimulus.

Previously, those who presented with pain that either persisted beyond the expected healing time after trauma or reported pain that far out weighed the actual bodily harm may have been dismissed as being malingerers or have psychosomatic pain.

The apparent dichotomy between reported pain and tissue insult and the work of Melzack and Wall has increasingly led researchers to investigate the role of the central nervous system in initiating and/or maintaining chronicity in a number of clinical pain syndromes. Psychological factors are now seen as an integral component of pain processing and are the subject of much investigation of their role in not only maintaining chronic pain but also as the target of therapeutic intervention (Gifford 1998).

1.5 Investigating pain

Variability in the sensory manifestations and both temporal and behavioural characteristics render investigation of clinical pain complex (Woolf 1997); the individual and subjective nature of chronic pain making it difficult to define, quantify and hence ultimately treat effectively.

Experimental pain in healthy volunteers is easier than clinical pain to both control and standardise therefore is appealing to pain researchers (Arendt-Nielson & Svensson., 2001). This very standardisation, however, is perhaps also what contributes to the limitations of experimental models when extrapolating results to a clinical population.

How similar an experimental model tested in healthy volunteers is to a clinical condition in a patient population has been questioned (Staahl et al., 2006).

Reliable reproduction of clinical pain syndromes however has proved difficult therefore application of experimental pain in a clinical population may be a useful compromise.

Although frequently quoted that cutaneous pain and muscle pain differ in their respective psychophysical properties (Svensson et al., 1997); direct comparisons of respective differences within the literature are scarce (Witting et al., 2001).

1.6 Soft tissue pain

Pain in the muscles is a common phenomenon affecting most people on occasion (Bennett 2007), usually occurring because of injury or strain, and settling down quickly once the affected muscle is rested. Myofascial pain is a common condition characterised by trigger points in a muscle which cause spontaneous pain and are painful to light compression (Yap 2007). A recent survey suggests up to 20% of the adult population in Europe, particularly women and the elderly may be affected by chronic musculoskeletal pain (Breivik et al., 2006). Although it is usually self-limiting, it is a causative or contributing factor in up to 30% of patients with chronic pain attending a pain clinic (Bennett 2007). Muscle pain can be difficult to localise, is variously described as cramping or aching in nature, and is frequently referred to other tissues (Mense 2003).

Svenssen et al., (1997c) suggest a commonality of central processing for muscle and skin pain as nociceptive afferents from both skin and muscle converge onto wide dynamic range neurons in the spinal dorsal horn, although other researchers emphasise the differences in skin and muscle pain processing throughout the central nervous system. This is also evident when comparisons are made between visceral and skin pain, with nociceptive responses from both visceral and cutaneous tissue converging on the dorsal horn such that the spinothalamic pathway contains neurons which respond to noxious events in both types of tissue (Strigo et al., 2003). These researchers did demonstrate a difference in cerebral processing between visceral pain (an increase in primary somatosensory and motor cortical processing) and skin pain (increased activation in anterior cingulate cortex and ventrolateral prefrontal cortex).

Muscle nociceptors are classified as group III (with similar properties to Aδ fibres, including a thin layer of myelination) and Group IV (unmyelinated and with similar properties to C-fibres, Graven-Nielsen and Mense, 2001). Group IV fibres have been shown to end exclusively in free nerve endings, while group IV fibres, in addition to free nerve endings, also have some other receptors such as paciniform corpuscles (Graven-Nielsen and Mense, 2001). Group III fibres have a conduction velocity of 2.5 – 30 ms^{-1} (measured in cats) whereas the velocity of Group IV fibres is slower at 2.5ms^{-1}; these figures are comparable to the conduction velocities of Aδ and C-fibres respectively.

In addition to differences in the physical properties of peripheral afferents mediating noxious stimuli from skin and muscle, differences are also reported in the processing of impulse transmission at various levels of the central nervous system. Small diameter muscle afferents do not terminate in lamina III of the dorsal horn where cutaneous afferents are numerous (Molander and Grant, 1987; Mense 2003). Central terminals of unmyelinated muscle afferents cover a larger area in the dorsal horn than those of cutaneous afferents while their density is lower (Ling et al., 2003).

In the thalamus, muscle nociceptor input into the ventrolateral and dorsal nuclei are more pronounced than from the skin (Gholami et al., 2006). The descending dorsal column pathways appear to impose a stronger inhibitory action on dorsal horn neurones processing noxious input from muscle than skin (Yu and Mense 1990). These anatomic and physiological differences suggest a functional differentiation that may sub serve specific behavioural patterns. For example, stimulation of group III and IV afferents, using hypertonic saline injection, has been shown to facilitate motoneurone activity, while depressing motor cortex activity (Martin et al., 2008), arguably allowing the muscle to rest while healing takes place.

Much of the research on muscle pain has been done with animal models, with the limitation of results not always being applicable to clinical pain conditions.

1.7 Differences in clinical phenomenology

Referral of pain is a typical characteristic of muscle pain (Capra and Ro 2004); for example injection of hypertonic saline into temporalis muscle elicits referred pain in the teeth (Jensen and Norup 1992) and into tibialis major causes ankle pain (Graven-Nielsen et al., 1997a).

In contrast noxious stimulation of the skin leads to secondary hyperalgesia characterised by a stinging, burning sensation and a visible, local "flare" surrounding the injured area of skin (Geber et al., 2007). This flare occurs because of the dual action of stimulated c-fibres – first, to activate the pain network, and secondly to indirectly stimulate the release of neuropeptides.

Easy access to the skin and controllability increases the appeal of cutaneous models of experimental pain, however how closely these models reflect clinical pain has been particularly questioned (Staahl et al., 2006). Experimental muscle pain has been shown to closely mimic clinical musculoskeletal pain in both subjective quality and motor performance (Arendt-Nielsen et al., 1996) and in response to pharmacological intervention (Curatolo et al., 2000). As such may provide a better model of clinical pain for investigation.

Various methods have been developed to evoke experimental pain in the skin and in muscles in healthy volunteers and animal studies. They typically have a rapid, short mode of action and no lasting effects, so are considered safe for use in experimental models. A constraint of many studies comparing muscle and skin pain lies in the use of different methods to stimulate a painful response (e.g. Svenssen et al., 1997a) using electrical stimulation to elicit muscle pain and laser stimulation to elicit skin pain).

1.8 Brain imaging investigations of pain

Advances in brain imaging techniques have allowed the central determinants of pain to be investigated. Although differences are observed, activation in multiple discrete cortical and subcortical areas has been observed dispelling the belief that the cortex is not involved in pain processing (Treede et al., 1999). These consistently include SII, thalamus, anterior cingulate cortex (ACC) and insula (Peyron 2000). Less consistent but still frequent areas where activation during a painful stimulus has been reported include SI / MI, prefrontal cortex (PFC), cerebellum and basal ganglia (Apkarian 2005). Areas even less frequently reported include amygdala and hippocampus.

Manipulation of various parameters including attention (Brooks et al., 2002, Bantick et al., 2002), intensity (Derbyshire et al., 1997), unpleasantness (Rainville et al., 2002), expectation (Plogaus et al., 2003) and hypnotic induction (Derbyshire et al., 2004) will all influence areas of activation observed.

Studies utilising experimental phasic cutaneous pain in healthy volunteers dominate the imaging and pain literature; investigations of the central nervous system during experimental muscle pain are sparse; perhaps due to perceived controllability, particularly in relation to the ability to control phasic muscle pain. There is also a lack of brain imaging studies investigating clinical pain syndromes although these are increasing.

Chronic pain affects a significant proportion of the population, causing distress and disability to the sufferer in addition to being a socio-economic burden in terms of both health care and welfare. Muscle and soft tissue pain conditions are amongst the most commonly seen, but under-investigated; epidemiological studies suggesting the incidence being 2 – 9% of the population at any given time (Mense 2003). Despite this there is a paucity of imaging research investigating musculoskeletal pain.

The author's clinical experience of treating chronic musculoskeletal pain and the paucity of research utilising human experimental muscle pain models in brain imaging studies provided the impetus for the studies described in this thesis.

The thesis will address the following questions:

1. When experimental muscle and cutaneous pain are induced in healthy volunteers utilising the same methodology and intensity matching, are the psychophysical properties sufficiently different to enable clear distinction between pain originating in the two tissue types.
2. Are differences in psychophysical properties of experimental muscle and cutaneous pain induced in healthy volunteers reflected in observed brain activation patterns?
3. Does the presence of a pre-existing clinical pain condition (tennis elbow) enhance or negate differences observed in healthy volunteers.
4. How does clinical pain compare to experimental pain induced in contralateral but similar tissue.

1.9 Hypotheses

Hypotheses are based primarily on the expected differing behavioural response, the knowledge that muscle pain is consistently considered to be more unpleasant than cutaneous pain (Rainville et al., 1992, Svensson et al., 1997a) and what is known from the small number of imaging studies utilising muscle pain.

The human brain must have the ability to differentiate between when 'fight / flight' is appropriate and when a 'stop and protect signal' in fact is required.

Cutaneous pain predominantly arises from an external threat; whether that is pinprick, heat, cold or chemical. The response is fight, i.e. to defend oneself against attack or flight or in other words to distance oneself from the danger. Important information that the brain must process is the source, site and severity of damage therefore it is hypothesised that the lateral or direct pain pathway is likely to predominate.

Conversely pain arising from deep tissue, including muscle is likely to arise from an internal threat e.g. damage, disease or infection of deep tissue and initiates an

altogether different behavioural response – one of stop and protect i.e. to reduce activity, evaluate, prevent further damage and facilitate healing. In this scenario fight is of no purpose as the 'fight' per se is internal and flight would involve the use and therefore potentially further damage to the already injured / diseased tissue. Whilst sensory discriminative components will also have a role, it is proposed that the evaluative components are of greater importance.

Furthermore pain arising from muscle tissue will typically have a less apparent origin that the external insult that results in cutaneous pain hence it is expected that muscle pain will result in greater activity in cognitive processing and may induce a more fearful response. Therefore it is hypothesised that the medial pain pathway will predominate.

Through comparisons with the literature the hypothesis that experimental muscle pain rather than experimental cutaneous pain is more reflective of clinical pain will be tested.

Imaging predictions

1. Widespread activation will be observed in the 'pain matrix' during both muscle and cutaneous pain conditions.
2. Greater / more extensive activation will be observed in SI, posterior insula and lateral thalamus during cutaneous pain when compared to muscle pain reflecting greater activity in the lateral pain pathways.
3. Greater more extensive activation with be observed in rostral ACC, rostral anterior insula and the prefrontal cortex during muscle pain compared to cutaneous pain reflecting greater activity in the medial or 'affective' areas of the pain pathways.

Thesis summary

Chapter 2

Chapter 2 presents a review of the literature in relation to three key areas of pain; experimental models of pain, the role of the brain in pain processing in response to experimental pain and role of the brain in relation to clinical pain processing.
The chapter serves to introduce the background for the four experiments described later in the thesis.

Chapter 3

Chapter three presents the methodology and results of Experiment One; a laboratory based investigation of the psychophysical properties of hypertonic saline induced pain in muscle (MS), intradermal (ID) and subcutaneous (SC) tissues.
The key aims of experiment one was (i) determine whether the psychophysical properties of deep and superficial pain differ when pain of similar intensity is induced using the same method of noxious stimulus and (ii) to investigate the applicability and reliability of the hypertonic saline model to consider its use in the planned fMRI studies.

Chapter 4

Chapter four begins with an overview of fMRI and methodology providing the background for the fMRI methods applied in three experiments described in the thesis.
The chapter then continues with the methodology specific to experiment two; fMRI undertaken in 18 healthy volunteers during hypertonic saline induced muscle and cutaneous pain. The results of this experiment are then presented. The chapter concludes with a brief discussion of the findings.

Chapter 5

Chapter five describes two experiments carried out in three patients with a clinical diagnosis of lateral epicondylitis (tennis elbow).

The first experiment, in which all three patients participated, replicates the methods employed in the healthy volunteer experiment described in Chapter 4.

The second experiment, in which two patients participated, was also an fMRI study in which patients were subjected to both innocuous and noxious pressure stimuli to both the affected and unaffected arms.

The results of the experiments are presented then the chapter concludes with a brief discussion of the findings.

Chapter six

This final discussion chapter begins with a summary of the findings, followed by a review of associated methodological issues and further discussion and interpretation of the results of each experiment presented in the thesis in turn.

The chapter concludes with a discussion regarding the implications of the findings of all three experiments and makes recommendation for future investigations.

This chapter reviews the literature with reference to what is currently known in regards to three key areas related to pain; experimental models of pain, the role of the brain in pain processing in response to experimental pain and role of the brain in relation to clinical pain processing.

The chapter begins with experimental pain, reviewing methods of induction (electrical, thermal, ischaemic, pressure, chemical), reliability and validity of the models, similarities and differences between models and tissue types and finally measurement.

There follows a brief overview of imaging techniques available; then continues to describe the so called 'pain matrix' and discusses the main components of the pain matrix. Studies involving experimental muscle pain are then specifically reviewed and an overview of cognitive and effective modulation of brain activity presented before a discussion on the functional division of pain pathways into medial and lateral components is presented.

Finally the chapter considers the literature that has employed brain imaging techniques to investigate clinical pain syndromes.

2.1 Background: Experimental models of pain

Progress with regards to management of pain is contingent upon treatment being targeted at the mechanism(s) that operates to produce a patient's symptoms (Woolf and Mannion, 1999). Variability in the sensory manifestations and temporal and behavioural characteristics however, render investigation of clinical pain complex. Clinical pain can rarely be standardised; some clinical conditions are episodic with intermittent quiescent periods of variable duration. Others may report a near constant background pain with frequent or infrequent exacerbations of varying duration and intensity. Clinical pain may be stimulus dependent or independent; be affected by temperature, circadian rhythms, menstrual cycle in women and a range of psychological factors. The latter includes anxiety and depression levels, previous pain experience, and situational and environmental factors (Keefe et al., 2005). In summary investigation of clinical pain is complex particularly when investigating effectiveness of a therapeutic intervention.

Experimental studies in healthy volunteers may therefore be useful, (Arendt-Nielson & Svensson 2001). How closely experimental models reflect the clinical situation however has been questioned (Petersen and Rowbotham, 1999).

A number of experimental models, particularly cutaneous pain have been described in the literature employing a variety of techniques utilising thermal, electrical, mechanical, chemical or ischaemic stimuli. Although these have added much to our knowledge base of the physiology of pain the models, particularly cutaneous ones are not without limitations. Cutaneous models however are particularly well developed; a possible reason for this being the ease of access to skin to introduce noxious stimuli.

The cutaneous models do have many advantages – ease of application, repeatability and ability to switch pain 'on' and 'off'. However these very advantages also contribute to their limitations. Typically studies have been of a block design, utilising phasic pain stimuli; clinical pain however rarely happens in 15 second bursts, nor is it often limited to cutaneous tissue. However experimental models do exist which produce a stimulus that is more tonic in

nature therefore arguably more closely aligned to the clinical situation (Mørk et al. 2003).

Arendt-Nielsen and Sumikura (2002) defined the 'ideal' experimental pain stimulus in humans as having the following characteristics:

- Non-invasive; producing no tissue damage
- Specificity – the stimulus stimulates the nociceptive system specifically rather than sensory-motor systems
- Sensitivity – the ability to measure pain within a range that is both ethically and physiologically acceptable to the participant
- Measurable – showing a relationship between stimulus and reported pain intensity
- Variable - the response shows an inherent variability from zero to maximum tolerable levels
- Reproducible – the stimulus produces a response that is repeatable over a number of repetitions and time

2.2 Experimental models; methods of pain induction

In order to elicit peripheral pain, small diameter afferent fibres must be activated. These fibres are classified as either thin myelinated (Aδ in cutaneous, group III in muscle) with fast conduction velocities (Meyer et al 2006) or unmyelinated (C-fibre in cutaneous, group IV in muscle) or further classified according to their conduction velocities.

2.2.1 Electrical stimulation

Electrical stimulation has been frequently employed in both human and animal models of experimental pain (Handwerker and Kobal 1993) to evoke either cutaneous or muscle pain (Graven-Nielsen and Mense 2001).

Nociceptors are stimulated by the insertion of fine electrodes into either the intradermal / subcutaneous tissue or the belly of a muscle; the later may result in electrical stimulation of the electrodes causing painful twitching of the muscle (Svenssen et al,. 1997); this twitching can itself introduce a confound into investigations employing this method. Geber et al., (2007) however did demonstrate good test-retest reliability using an intradermal model of electrical stimulation in healthy volunteers.

Onset and offset of electrical stimulation is well defined (Gregersen et al., 2007) which can eliminate the latency to stimulation of afferent fibres that may occur with other pain induction methods. Parameters of electrical stimulation apparatus may be adjusted thereby allowing a variety of stimulation patterns to be delivered which provides some degree of selectivity in exciting different primary afferent neurons and hence evoking different types of pain

Electrical stimulation of the nociceptive pathway however, bypasses nociceptor transduction, depolarising the primary afferent fibre directly. Furthermore electrical stimulation acts on a broad spectrum of afferents both non-painful (group I and II fibres) and painful (group III and IV fibres) with lower stimulation intensities required to activate thick myelinated (non-nociceptive) afferents than unmyelinated nociceptive fibres. Electrical stimuli therefore are not specifically nociceptive (Thunberg et al., 2005) however a number of studies have employed electrical stimulation to the cutaneous (Svensson et al., 1997), muscle (Vecchiet et al., 1999, Arendt Nielsen et al., 1997) and visceral (Drewes et al., 2006) tissues.

The overriding problem associated with electrical stimulation therefore is its inherent non-specificity; the threshold for electrical stimulation is related to the sensory fibre diameter therefore at intensities sufficient to excite small diameter fibres, large diameter fibres will also be excited. These include non-noxious sensory fibres and also muscle efferent fibres, potentially resulting in local muscle contraction adding a further confounding factor.

2.2.2. Thermal pain

2.2.2.1 Contact heat

A contact Peltier thermode is placed on a body part and temperature is increased at a fixed rate, typically 1^0C / second activating both Ad and C fibres (Hughes et al., 2002). Heat pain threshold, tolerance and suprathreshold levels may be tested.

Temperature increases induced in the skin are dependent on a number of factors including reflectance, transmission, and absorption of the epidermis (Bromm and Treede 1991, Le Bars et al., 2001) which gives rise to variability between individual subjects. The advantages of contact heat include controllability of the pain induced allows the pain to be switched on and off with a rapid onset and offset time.

Thermode application activates small myelinated Adelta and unmyelinated C-fibres however also low-threshold mechanosensitive afferent fibres, which can modulate the spinal transmission of nociceptive and thermal information; thereby potentially excerpting an inhibitory influence on the pain mechanisms (Staahl and Drewes 2004).

2.2.2.2 Laser induced heat

Cutaneous pain can be evoked using a high energy CO_2 laser beam to direct radiant heat to a small area of skin (Svenssen at al., 1997), causing a stinging and burning sensation. A limitation of laser beams is that, because it induces rapid heating of the skin, it is likely that, as well as stimulating C-fibres, there is also the possibility that sensitive mechanoreceptors will also be excited (Handwerker and Kobal (1993).

CO_2 lasers operate in the far infrared range (10·6 μm), hence avoid some of the problems associated with contact heat (Plaghki and Mouraux., 2003). Temperature increases are extremely fast; activating nociceptors within a few milliseconds. As skin absorption is almost 100%, energy is confined to the upper cutaneous layers i.e. where nociceptors are located.

CO_2 laser has the further advantage of allowing selective stimulation of specific fibre classes; stimulation parameters may be adjusted to target small myelinated and unmyelinated fibres without concomitant activation of Abeta fibres or C-fibres without Adelta fibres (Plaghki and Mouraux., 2003; Magerl et al., 1999; Tran et al., 2001).

It has been suggested however that as laser stimulation produces a synchronised fast onset it may feel subjectively different from other more natural pain stimuli (Kupers and Kehlet, 2006).

2.2.2.3 Cold pain

The cold pressor test evokes pain by plunging an extremity – either the foot or the hand into a receptacle of ice - saturated water (0-2°C) (Curatolo et al., 2000). The exact mechanism by which the pain is mediated is unclear. Initial immersion induces cutaneous vasoconstriction hence cold receptors will indeed be stimulated however the cold also induces a marked vasoconstriction (Wilson et al., 2007) resulting in ischaemic pain. The cold pressor test has also been shown to be unreliable in clinical testing (Blasco and Bayes 1988).

Cooling and therefore cold pain is now more typically induced by application of a Peltier thermode and decreasing the temperature in the same manner as above (Liem et al., 2005). Cooling may also be achieved by topical application of ice, alcohol, menthol or ether (Staahl and Drewes 2004).

One group of investigators have explored heat as a noxious muscle stimulus through injection of isotonic saline at temperatures of 48°C (Graven Nielsen et al., 2003). Pain was induced however this was only mild (peak VAS 3.2). Furthermore there are methodological issues associated with maintenance of saline temperature and safety issues regarding maximum temperature required to induce pain.

In summary thermal pain models provide a valid and reliable model of pain induction for cutaneous pain. A limitation in terms of comparison however is the lack of an appropriate comparator model in muscle.

2.2.3 Ischaemia induced pain

Ischaemia-induced muscle pain is a long-standing method induced by the use of a tourniquet (Graven-Nielsen and Arendt-Nielsen 2003). This pain, involving the whole limb, is caused by deprivation of oxygen in all tissues distal to the tourniquet during the temporary (15-20min) occlusion of blood supply to the limb. It is followed by a variable degree of post ischaemic paraesthesia (Pertovaara et al 1984). The pain induced by the tight tourniquet is of similar severity to the ischaemic pain (Pertovaara et al., 1984). These features significantly reduce the applicability of ischaemic pain to study cutaneous and deep tissue pain separately. Ischemia has also been used in animal models of visceral pain (Ness and Gebhart, 1990) however is not considered an acceptable model for investigation of visceral pain in humans (Drewes et al., 2006)

2.2.4 Chemical pain

In an attempt to reproduce some of the features of clinical pain a number of chemical agents have been employed to induce pain – again in both cutaneous and muscle tissue. The most common of these agents is capsaicin (Witting et al., 2001).

Capsaicin, extracted from chilli peppers, is used either as a local application to evoke skin pain, or by injection – subcutaneous or intradermal to evoke skin pain, and intramuscular to evoke muscle pain (Capra and Ro 2004), producing moderate pain and inflammation.

Intradermal administration of capsaicin also causes secondary allodynia and hyperalgesia (LaMotte et al., 1991), its action mediated through vanilloid receptors (VR1) found on heat nociceptors (Tominaga et al., 1998)

Due to a prolonged period of action it may be avoided in human experiments requiring short durations of pain stimulus.

Concerns have also arisen regarding toxicity effects of injection of algesic agents; although a number have been tested in humans, their use is typically more limited to animal models.

2.2.5 Mechanically induced pain

Mechanical stimulation may be applied via a pressure algometer allowing standardised, measurable graded increases or decrease of pressure to be applied

evoking mechanical muscle (Graven-Nielsen and Arendt-Nielsen 2003) pain. This method of pain induction has been shown to yield good reliability (Nussbaum et al., 1998; Vatine et al., 1993; Antonaci et al., 1998). The later two groups also demonstrated no difference between dominant and non-dominant sides.

Mechanical stimulation however is again non-specific as low threshold mechanoreceptors in the skin are also stimulated (Handwerker and Kobal 1993) hence it may be difficult to differentiate the effect on muscle and skin separately. Kosek et al., (1999) demonstrated that skin sensitivity did affect pain pressure thresholds in a double- blind investigation employing EMLA cream, a local anaesthetic. Pre – application of a topical anaesthetic could perhaps be employed to negate the effects of cutaneous stimulation however the depth to which EMLA penetrates and therefore the reproducibility of this method has been questioned (Kosek et al., 1999).

The mechanical properties of the gastrointestinal tract are important for its function as a digestive organ and the gut contains mechanoreceptors at various locations in the wall (Ness and Gebhart, 1990). As such visceral mechanical models are particularly well developed, the most common of these being distension, typically via an inflatable balloon either rectally or via the oesophagus (Drewes et al., 2006).

2.2.6 Hypertonic saline induced pain

Injection of hypertonic saline has been employed extensively in both animal and human muscle pain studies. Kellgren (1938) first wrote about its use and the technique has been widely investigated since. Intramuscular injection of hypertonic saline (HS) is perhaps the most established technique of inducing experimental muscle pain, having been extensively studied with regards to psychophysical properties (Graven-Nielsen et al 1997). It has proved a useful tool in improving understanding about the peripheral mechanisms of muscle pain and been shown to closely mimic clinical musculoskeletal pain in both subjective quality and motor performance (Arendt-Nielsen et al., 1996). It has been less well utilised in cutaneous studies.

Injection of hypertonic saline causes a short lasting, reversible, moderate intensity pain which has been widely tested, has never been associated with serious complications and there are no reports of muscle toxicity (Marchettini et al., 1996).

Despite the long history of investigators employing HS as a model of experimental pain, it is only in recent years that investigations have revealed the probable mechanistic basis for the nociceptive effects of HS.

In vitro and in vivo experiments show that hypertonic saline at 2 – 5 % concentration stimulates C-fibre afferents (Pedersen et al 1998, Alessandri-Haber 2005); providing the theoretical basis for its use in studies of experimental skin and muscle pain (Alessandri-Haber et al., 2005).

It has been postulated that the pain-inducing effect of hypertonic saline is mediated through changes in local osmotic pressure to which a number of ion channels located in nociceptors respond. The receptors with the required properties are members of the TRPV family (TRPV1, TRPV2, TRPV4, and the stretch-inactivated channel, SIC as well as TREK-1, a two pore voltage-gated K+ channel). Importantly, TRPV1, TRPV4, SIC and TREK-1 are found in small, unmyelinated sensory fibres (Alessandri-Haber et al 2005, Schumacher et al 2000, Liu et al 2007, Alloui et al 2006).

TREK-1 is co-localised with TRPV1, the latter able to transduce thermal, mechanical and hyperosmolar stimuli (Alloui et al 2006). To date the strongest case for this action comes from studies conducted on TRPV4. Hypertonic saline injected intradermally in the paw of the rat induced concentration-dependent pain behaviour in them (Alessandri-Haber et al 2005). PGE2 enhances this response at low concentrations of hypertonic saline but not at higher concentrations (10%). Because only very small volumes of hypertonic saline are needed, the changes in local tonicity are likely to normalise quickly lessening the capacity of the saline to maintain nociceptor activation.

Initial nociceptor depolarization, via activation of SIC or TRPV4, however may induce neurogenic release of glutamate and neuropeptides (Hua et al., 1986 and Lawand et al., 2000) which in theory, if injections are give repeatedly may lead to a prolonged or heightened response (Coggeshall and Carlton, 1997). Alternatively, prolonged stimulation may lead to habituation.

A mechanosensitive non-selective cation channel, known as stretch-inactivated channel (SIC), has also been suggested as a potential transducer for HS induced pain (Schumacher et al., 2000). SIC is a variant of TRPV1 that is activated by cell shrinkage and inhibited by cell swelling (Suzuki et al., 1999). As SIC is expressed in small diameter neurons in the DRG (Schumacher et al., 2000), changes in osmolarity in the extracellular fluid environment around nociceptive terminals can activate SIC upon cell shrinkage and depolarize nociceptors.

Therefore, it is suggested that SIC may function as mechanical transducer mediating muscle tenderness, a symptom frequently seen in patients with chronic musculoskeletal pain. Hence HS may indeed be a useful model of clinical MSK pain conditions.

Hypertonic saline has been employed to induce experimental muscle pain in a controlled manner in healthy volunteers (Graven-Nielsen et al., 1997) and the technique has been extensively investigated for psychophysical properties, particularly in relation to referred muscle pain. Induced pain has been shown to be dependent on infusion rate, volume and concentration of the HS introduced (Jensen and Norup, 1992).

Graven Nielsen and colleagues have also developed a model of muscle pain induction which produces a longer lasting pain response through continuous infusion of minute volumes. Whilst this has proved useful in investigations of psychophysical properties (Graven Nielsen et al., 1997) and tests of drug effectiveness (Curatolo et al., 2000), the infusion model is less suitable for investigations requiring pain induced to be of more limited duration; for example in a scanning environment.
There is little evidence however that it has been used in cutaneous pain.

Observations from studies investigating motor effects of (Arendt-Nielsen et al., 1996) and pharmacological response to (Curatolo et al., 2000) HS also indicate that pain elicited by HS injection / infusion reflects acute, clinical muscle pain. Its use has never been associated with serious complications and there are no reports of muscle toxicity (Marchettini et al., 1996). As the pain lasts for several minutes it allows for an accurate collection of the subject's impressions of sensory and motor effects, including the phenomenon of referred pain.

Taken together the evidence would suggest that hypertonic saline can be injected safely and repeatedly, induces a relatively short-lasting reversible pain and may serve as a reasonable surrogate model of clinical musculoskeletal pain in healthy volunteers

2.2.7 Combined experimental models

Whilst the controlled environment of experimental pain and healthy volunteers has great appeal, there is recognition of the limitation particularly in that they tell us about physiology and not pathophysiology.

A surrogate model of clinical pain is therefore desirable. Petersen and Rowbotham (1999) developed such a model; a novel method of pain induction involving pre-sensitisation of cutaneous tissue with heat prior to topical application of capsaicin cream. This produces a thermal hyperalgesia i.e. a lowering of the threshold of heat pain. The model is said to replicate heat allodynia seen in many clinical neuropathic pain conditions.

Capsaicin-induced heat allodynia is thought to depend largely on the activity of C nociceptors because it persists when the conduction of A fibres is selectively blocked (Torebjörk et al., 1992). There is however some evidence of the involvement of a unique subgroup of Aδ nociceptors by the capsaicin-induced heat allodynia (Ringkamp et al., 2001).

Some C nociceptors are also exclusively responsive to heat following the application of irritants, such as capsaicin or mustard oil (Handwerker and Kobal, 1993).

Heat allodynia is also distinct from 'normal' heat pain in that different spinal mechanisms are thought to be involved; heat allodynia involving the sensitisation of spinal cord projection neurons, characterised by the enhancement of their response to heat and enlargement of their receptive fields (Woolf and Salter, 2000).

It is suggested therefore that heat allodynia following chemical irritants may involve the recruitment of a class of nociceptors that are functionally distinct, conveying information regarding pathophysiology of the tissue rather than the actual or potential threat of heat damage.

Since its initial development the heat – capsaicin model has been extensively tested in experimental studies, drug efficacy investigations and brain imaging studies. The model shows initial promise, appearing to more closely reflect the psychophysical properties and cerebral correlates of heat hyperalgesia observed in some patients with neuropathic pain.

2.3 Interpretation of the measured response

Pain is a subjective sensation; as such it is not possible therefore to directly measure, however certain components can be measured, which combined, provide information regarding the pain experienced by an individual.

Perceived intensity and quality, both unpleasantness and descriptive semantics are the main aspects frequently measured. Combinations of these factors provide some information with regards to the working of the nociceptive system. Under experimental conditions on healthy volunteers, stimulus modality, duration and intensity can be defined and hence controlled.

Visual analogue scales (VAS) are typically employed to take a measure of pain intensity; consisting of a 10cm long line, the extremes are labelled to represent the extremes of a potential painful response. That is the left anchor is typically labelled 'no pain' whilst the right anchor is labelled 'pain as bad as could be' or 'worst possible pain' (Jenson et al., 1986). Visual analogue scales have been found to correlate well with other measures of pain self report (Jensen et al., 1989; Paice and Cohen, 1997) and observed behaviour responses (Gramling and Elliot, 1992.) Ahles et al., (1984) also demonstrated that VAS measurements are distinct from measures of other subjective components of pain experienced.

Whilst visual analogue pain scales provide a reliable measure of perceived pain intensity they fail to capture the multidimensional aspect of pain. The McGill Pain Questionnaire (MPQ) therefore provides a method of investigating these (Melzack, 1975). The scale includes descriptive words of sensory, affective and evaluative aspects of pain; whilst the usefulness of the scale has been demonstrated in a number of clinical and some laboratory based investigations, its length and therefore time taken to complete preclude its usefulness in experimental pain studies investigating relatively short-lasting stimuli.

A Short-Form MPQ (SF-MPQ) was subsequently developed by the originator of the MPQ (Melzack, 1987), and recommended for use in specific research and clinical settings when the time to obtain information is limited (Melzack and Katz 2006). The SF-MPQ consists of 15 descriptors of pain, 11 from the sensory and 4 from the affective categories of the MPQ. Words were selected on the basis of endorsement by patients with a variety of acute, intermittent, and chronic pains (Melzack and Katz, 2001).

Sensory and affective factor scores have been show to correlate highly with those from the MPQ (Melzack 1987) and factorial validity shown to have good internal consistency estimates for both the sensory and affective dimensions (Wright et al., 2001)

2.4 Comparisons of cutaneous and muscle pain.

The suggestion that muscle and skin pain are different is not a new one, Thomas Lewis having written on the subject of perceptual and behavioural differences (Lewis, 1942). Pain of cutaneous origin is typically described as possessing a sharp, prickly or burning sensation and is easily localised. Behaviourally the expected response is one of fight or flight i.e. removal of oneself from the source of the pain and therefore limit further danger or damage. Muscle pain on the other hand is frequently described as dull, aching, throbbing sensation which is difficult to localise, and may evoke a characteristic passive coping manner.

Despite the prevailing view that pain arising from cutaneous and muscle pain show different characteristics, direct comparisons in the literature of pain from these two tissue types, evoked by the same stimulus are rare.

Rainville et al (1992) undertook a comparison of the psychophysical properties of four different stimulus modalities; contact heat, electrical, cold-pressor and ischaemic exercise induced pain. They clearly demonstrated that the psychophysical properties of a painful response relative to the intensity of a stimulus vary according to the type of stimulus modality employed. The results, they suggest indicate ischaemic exercise and cold pressor pain may be a better model of clinical pain having higher levels of unpleasantness induced whereas the lower levels of unpleasantness of contact heat may suggest this model is better for dissociating sensory discriminative aspects of pain.

Svensson et al (1997) also investigated stimulus response curves of cutaneous and muscle pain; reporting that although these were similar in form, unpleasantness / intensity ratios of muscle and cutaneous pain were different; muscle pain having a relatively higher ratio than cutaneous pain. Interestingly both of these groups of investigators report the intensity component of the ratio as a function of stimulus intensity rather than response. Although increasing intensity of pain does indeed follow increasing intensity of stimuli, the response is not necessarily linear.

Caution therefore should be taken when the authors interpret their findings as evidence that pain arising from muscle tissue is more unpleasant than that from cutaneous stimulation.

Witting et al., (2001) also undertook a direct comparison of cutaneous and muscle pain using capsaicin injections. The focus of their investigation however was on differences in referred symptoms which they duly found; referred sensations were frequently reported in muscle but not cutaneous pain.

A direct comparison of psychophysical properties of subcutaneous, intradermal and muscle pain induced by hypertonic saline has not been undertaken, to the best of the author's knowledge.

2.5 Summary of experimental pain

Pain, in common with any form of somatic sensation, is typically described in terms of sensory, cognitive and affective aspects. Sensory aspects generally include location, magnitude and spatiotemporal properties. Affective components include the hedonic aspect – i.e. the degree of unpleasantness of the sensation and a motivational aspect – producing the motivation to limit the experience of the noxious stimulus (Sewrads et al., 2002).

It has been argued that the hedonic and sensory aspects of somatic sensation are represented and processed in parallel but distinct pathways throughout the nervous system and may reflect differences in psychophysical properties of cutaneous and muscle pain.

The sensory-discriminative and affective-motivational aspects of pain differ according to the forebrain targets of ascending pathways (Melzack and Casey 1968; Price 2000 and Hunt and Mantyh 2001). Therefore, the central processing of cutaneous and muscle pain, according to the types of nociceptors and spinal projections mediating these sensations may also differ.

In order for a direct comparison of either the psychophysical or cerebral properties of muscle and cutaneous pain to be undertaken; pain must be elicited via the same method of induction. Reviewing the evidence suggests that hypertonic saline can be injected safely and repeatedly into either muscle or cutaneous tissue, induces a relatively short-lasting reversible pain and therefore would appear to be the most appropriate model to utilise if indeed aim is to carry out a comparative study of relative psychophysical properties of the se two tissue types.

2.5. Background: Neural processing of pain

Advances in brain imaging techniques have led to a number of methods becoming available for the safe, non-invasive study of both normal and pathological pain processing in humans. Techniques include electroencephalographic dipole source analysis (EEG), magnetoencephalography (MEG), positron emission tomography (PET), single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI). Each method has inherent strengths and weaknesses in relation to sensitivity, spatial and temporal resolution however all provide a utility for measuring either directly or indirectly neuronal activity.

Electrophysiologic techniques provide direct measurements of neuronal activity with millisecond temporal resolution therefore allow the means to study dynamic nociceptive processing. However relatively poor localization in the brain to the origin of the signal is a significant weakness.

PET provides the means to measure both metabolic and neurochemical aspects of brain processing and allows for the identification and investigation of the roles of neurotransmitters and receptor systems involved in acute and chronic pain. As PET requires injection however of a radioactive tracer, dose restraints do limit the number of scans that may be acquired.

FMRI measures metabolic and haemodynamic responses to neuronal activity and provides better spatial and temporal resolution than PET for localizing brain

regions that are activated during pain processing, and for probing possible functional connections between activated regions. A further important advantage of fMRI is that due to the non- requirement for radioactive tracers there is not the same constraints placed in terms of repeating scans allowing longitudinal e.g. pre and post intervention studies to be undertaken.

2.5.1 The Pain Matrix

Melzack (1989) first proposed the idea of a pain 'neuromatrix' in an attempt to explain the possible mechanisms underlying phantom limb pain. He put forward his idea that phantom pain may be mediated by a network of neurons, the 'neuromatrix', involving three major neural circuits in the brain and incorporating the sensory, cognitive, affective and motor aspects of pain.

Subsequently, functional brain imaging studies have reported a widely distributed set of cortical and sub-cortical brain areas that are activated during a variety of experimental pain conditions. Despite some inconsistencies between studies, often attributed to differences in study design, a fairly robust set of structures considered to play a significant role in pain have become collectively know as the 'pain matrix' rather than Melzack's (1997) 'neuromatrix'. It is however accepted that the term has evolved from Melzack's original proposal (Derbyshire, 2000)
The pain matrix consistently includes primary and secondary somatosensory cortices (SI and SII), Anterior Cingulate Cortex (ACC), insular cortex, primary motor and premotor cortices, prefrontal cortex (PFC), and thalamus in addition to posterior parietal cortices, amygdala, basal ganglia, midbrain (periaqueductal grey matter), and cerebellum (Peyron et al., 2000).

A systematic review of pain imaging literature by Apkarian et al (2005) revealed the six most commonly reported areas to be ACC, SI, SII, insula, thalamus and prefrontal cortex (PFC).

2.5.1.1 Somatosensory cortices

Primary somatosensory cortex (SI)

The primary somatosensory cortex (SI), located in the posterior gyrus of the parietal lobe receives sensory information via projections from the ventral posterior medial (VPM) nucleus of the thalamus. Four cytoarchitectonical areas (Brodmann areas 3a, 3b, 1, and 2) have been defined in SI of primates and more recently humans (Kurth et al 2000); although the four areas are extensively interconnected, each contains a separate representation of the body surface and also a different functional role in sensory processing (Iwamura 1998). Areas 3b and 1 receive information from cutaneous sensory receptors whilst areas 3a and 2 receive proprioceptive information from joints and muscles.

Although its role in relation to pain processing continues to be a matter of debate, SI is thought to encode for intensity and location of pain despite containing only a relatively low number of nociceptive driven neurons (Svenson et al., 1997c), of which less than 10% receive input from deep (i.e. non cutaneous) tissue.

Nociceptive neurons in areas 3a, 3b, and 1 have been identified in macaque monkeys through electrophysiologic recordings (Kenshalo 1983). Ploner et al., (2002) however report serial activations in Brodmann areas 3b, 1 and posterior parietal cortex during touch but only activation of area 1 during nociceptive stimulation in humans suggesting that although SI appears to have a role in sensory-discriminative aspects of pain, the processing of pain would seem less hierarchically organised than tactile processing.

In a comprehensive review of pain imaging studies Apkarian found that over 50% of studies report SI activation in response to a painful stimulus. The lack of SI activation in the other pain studies may be in part due to insufficient intensity of pain produced (Svensson et al 1997), small body surface area stimulated (Peyron et al., 2000) or as suggested by Brooks et al., (2002) due to other affective or cognitive aspects of the individual and the environment of the experiment taking place.

Somatotopic reorganisation observed in phantom limb pain patients (Flor et al., 1997; Lotze et al., 1999) and Complex Regional Pain Syndrome (CRPS) patients (Maihofner et al., 2003) has also led to increased interest in SI. However although the extent of SI re-organisation has been shown to correlate with intensity of pain (Flor et al., 1997) and also reverse after successful rehabilitation (Maihofner et al., 2004) the exact role of SI remains unclear.

In summary multiple lines of evidence exist to indicate the involvement of SI in nociceptive processing; the predominant role being sensory discriminative. However the specific roles of each area during pain processing and its relationship with tactile system still need further investigation.

Secondary somatosensory cortex (SII)

The secondary somatosensory cortex (SII), located in the parietal operculum in the upper bank of the Sylvian fissure, receives projections from each of the four SI areas. SII neurones project to the insular cortex, in turn innervating regions of the temporal lobe believed to be important for tactile learning and memory (Mishkin 1979).

Despite the findings of Robinson et al (1980) that in macaques less than 3% of neurons recorded from SII respond to noxious stimuli, many human imaging studies consistently report activation of SII during experimentally induced pain (Peyron 2000). SII activation has been reported during contact heat in both PET (Talbot et al., 1991, Casey et al., 1996, Craig et al 1996, Rainville et al 1997, Svensson et al 1997c, Coghill et al., 1999) and fMRI (Davis et al., 1998, Apkarian et al., 1999; Becerra et al., 1999; Gelnar et al., 1999) studies.

MEG studies provide further evidence of SII activation during pain processing (Hari et al., 1997; Huttunen 1986; Kakigi 1995).

Lenz et al., (1998) recorded laser evoked potentials directly from SII via subdural electrodes implanted in three subjects suffering intractable epilepsy providing direct evidence of nociceptive input to the human parasylvian cortex.

Clinical observations in patients with SII lesions also point to SII having a role in pain perception; these patients demonstrating elevated pain thresholds to pin-prick stimulation (Greenspan and Winfield 1992, Greenspan et al., 1999). Ploner et al., (1999) in a single case study of a patient with an ischaemic lesion in the parietal operculum report the inability to recognise both the nature and location of an intense noxious stimulus. Despite this the patient did report an intense unpleasant sensation.

2.5.1.2 Insular cortices

The insular cortices, both posterior and anterior, have received much attention in the pain literature and are reported to be the most consistently activated regions during painful stimuli (Peyron 2000). Insula activation has been reported during a number of different noxious stimuli including painful heat (Casey et al., 1996, Coghill et al., 1994, Derbyshire et al., 1997), electrical stimulation (Svensson et al 1997, Oshiro et al., 1998, Niddam et al., 2002), pressure pain (Farina et al., 2002, Rolls et al., 2003) and both oesophageal (Aziz et al., 1997) and rectal (Mertz et al., 2000) painful distension. It is suggested that the insula may be seen as a site of sensory and affective integration (Brooks and Tracey 2007).

Functional and cytoarchitectonical differences in the insula

The insula may be split into anterior and posterior segments at both a cytoarchitectonical level and a functional level; posterior and anterior insular cortices change from granular in the posterior portion to agranular in the anterior portion. It also receives differential cortical and thalamic input along its length.
Anterior insula receives direct projections from the basal part of the ventral medial nucleus (VMb) of the thalamus and large inputs from the central nucleus of the amygdala with reciprocal projections.

The posterior insula connects reciprocally with the secondary primary sensory cortex (S2) and receives input from spinothalamically activated ventral posterior inferior (VPI) thalamic nuclei.

Functionally the posterior insula would seem to have a sensory discriminative role, contributing to localisation of pain; somatotopy during painful stimulus has been demonstrated (Dunckley et al., 2005, Brookes et al., 2005) and Ostrowsky et al., (2002) demonstrated pain in the contralateral hemibody during direct electrical stimulation of the posterior insula.

Anterior insula has been attributed with affective, motivational and memory aspects of pain. The area receives inputs from the ventromedial nucleus of the thalamus that contains predominantly nociceptive and thermoreceptive neurons specialised to convey information such as pain, temperature and itch (Craig et al 1995).

Activity has also been demonstrated in other non-painful but aversive states e.g anxiety, fear. Activation is also enhanced by attending to an aversive stimulus (Brookes et al., 2002) and stimulation within insula evokes painful experiences (Ostrowsky 2002). Greenspan et al., (1999) also demonstrated that damage to the insula results in a change in the affective quality of pain i.e. that pain is still felt but the associated unpleasantness is absent.

Schweinhardt et al., (2006) recently proposed a function subdivision of the anterior insula into rostral and caudal areas; from a review of the literature they reported that clinical pain was preferentially processed in the rostral anterior insula (rAI) whereas in experimental pain studies caudal anterior insula (cAI) predominated.

They suggest that the cAI may reliably encode the intensity of perceived pain whereas the rAI may uniquely reflect the processing of clinical pain; clinical pain having a more affective response that experimental pain. The authors do however acknowledge that perception of bodily states and also aversive (non-painful) stimuli also provoke rAI activation, as does modulation of experimental pain by attention. A further consideration should be that the experimental pain studies reviewed were all cutaneous models, which is considered less aversive than muscle pain.

2.5.1.3. Cingulate cortex

The cingulate cortex, situated in the medial aspect of the cortex, extends from the corpus callosum below to the cingulate sulcus above. Based on cytoarchitectonics and pattern of projections the anterior cingulate cortex (ACC) can be differentiated from the posterior cingulate cortex (PCC) (Vogt et al., 1992). Distinct also functionally, the ACC is considered to have an 'executive' role whilst PCC has an evaluative function (Vogt et al.,1992).

Anterior cingulate cortex (ACC)

The anterior cingulate cortex (ACC) has been divided into the perigenual (or rostral) anterior cingulate cortex (pACC) and midcingulate (MCC) cortex, with the MCC more recently also having been further divided in to posterior (pMCC) and anterior (aMCC) divisions (Vogt et al 2003). The aMCC is both anatomically and functionally distinct from the pMCC. The former is considered to be primarily concerned with fear and affect whilst the later is thought to be more involved with executive function.

Observations that the ACC is one of the structures most frequently activated in pain imaging studies suggest that the ACC has a key role in nociceptive processing (Derbyshire et al., 2000, Peyron et al., 2000). However there is also substantial variation in the exact location within the ACC of activation reported (Apkarian et al., 2005) which led Kulkarni and colleagues (2005) to suggest that the ACC may have both pain specific and non pain specific cognitive functions.

Mid cingulate cortex (MCC)

The MCC receives significant posterior parietal afferents (Vogt et al 1987) and contains the cingulate motor areas (CMAs) which project directly to the spinal cord and the motor cortices (Dum and Strick 1993, Van Hoesen et al., 1993). Functionally the MCC is involved in motor and non-motor response selection and behavioural change (Vogt 2003)

Although ACC is considered to code for unpleasantness, rather than intensity of a painful stimulus, discrete intensity associated areas have been identified within the mid cingulate cortex (Coghill et al., 1999, Derbyshire et al., 1997).

Perigenual (rostral) ACC (pACC)

Vogt et al., (1996) suggests that the affective or 'suffering' component of pain is primarily interpreted in the pACC; this area receiving significant input from the amygdala. Lenz et al., (1998) measured laser evoked potential directly from ACC (BA 24) in five patients with epilepsy via subdural grids demonstrating the presence of significant direct nociceptive input to this area. Evoked potentials were present bilaterally although stronger contralateral to the stimulus. ACC connections have also been reported with the prefrontal cortex, parietal cortex and the motor system (Posner & DiGirolamo, 1998). However some studies of experimental pain that have manipulated the affective component of the pain experience have failed to demonstrate pACC activation (Tolle et al 1999, Rainville et al 1997).

Conversely, Jones et al (2002) in a PET study where subjects selectively attended to unpleasantness during a painful laser stimulus demonstrated an increase in rCBF bilaterally in pACC. Whilst receiving the same stimulus but attending to location the increase in rCBF was found in the contralateral SI and inferior parietal cortices. Kulkarni et al., (2005) also demonstrated significantly greater increases in the pACC when subjects attended to the unpleasantness of a painful stimulus than when they attended to location of the same stimulus. The authors attribute the results to their use of localisation as comparator to unpleasantness rather than intensity suggesting that subjectively differentiating between intensity and unpleasantness was not as effective as differentiating localisation and unpleasantness.

Internally generated emotions activate pACC and significant increases in rCBF in the pACC during PET studies of psychiatric patients have been demonstrated in those diagnosed with obsessive compulsive disorders (Brieter 1996), phobic anxiety (Rauch et al., 1995), post-traumatic stress (Rauch et al., 1996) and mood disorders (Drevets et al., 1997) suggesting stress and /or anxiety may be closely associated with pACC and it is perhaps this element that is represented during pain studies.

Peyron et al., (2000) suggest that the relative lack of activation in the pACC demonstrated during experimental pain studies may be explained by the controlled situation leading to minimal stress or anxiety. The authors further point out that the opposite may be true for clinical pain where haemodynamic changes have been repeatedly reported in this area (see clinical pain section below).

Clinical lesion patients provide further evidence of a role for ACC in pain. After cingulotomy, patients with chronic pain report that they continue to have pain, which is "not particularly bothersome" (Folz and White 1962).
In patients with confirmed lesions of the cingulate gyrus a reduction in the affective response to pain (Ballantine et al., 1967, Davis et al., 1994, Pillay and Hassenbusch 1992) has been reported whilst the ability to localise noxious stimuli was retained. Results of such lesions however are variable with reported relief of chronic pain reported in 23% (Hurt and Ballantine, 1974) to 75% of patients (Folz and White, 1962).

Posterior cingulate cortex (PCC)
The PCC corresponds to Brodmann areas 23 and 31 and the restrosplenial cortex (RSC) posteriorly (Brodmann area 29) and is considered to have an evaluative role in assessing sensory input rather than initiating action in response (Vogt et al., 1992).

2.5.1.4 Thalamus

The thalamus, one of the two major subdivisions of the diencephalon, is an important link in the transfer of sensory information from afferent input from the periphery to a number of cortical areas. Originally considered to act simply as a relay station, a number of lines of evidence now point to a gating and modulatory role (Kandel et al 2000). Thalamus contains three major nuclear complexes that receive somatosensory input from ascending sensory pathways (Sewards and Sewards 2002); the ventroposterior complex, the posterior complex and the intralaminar thalamic nuclei. The ventrobasal complex is considered a 'specific' somatic relay, the nuclei receiving precisely organised input from DCN and the dorsal horn which is then relayed to the somatosensory cortex.

Thalamic activation in pain studies is again frequently but inconsistently found with studies reporting bilateral (Casey et al 1994, Coghill et al 1999, Vogt et al 1996,), ipsilateral (Adler et al., 1997, Derbyshire et al., 1998) and contralateral (Jones et al., 1991, Derbyshire et al., 1994, Becerra et al., 1999) activation.

Studies investigating attention and vigilance have shown bilateral increase in thalamic activation (Frederickson et al., 1995; Portas., et al 1998; Posner et al., 1994).

The evidence suggests therefore that the thalamus may have both sensory – discriminative and cognitive roles in pain processing which Peyron et al (1999) suggests may reflect a general 'arousal' response to nociceptive input.

2.5.1.5 Amygdala

The role of the amydala in pain processing has also been subject to increasing investigation. Nociceptive specific neurons have been observed in the central nucleus of the amygdala (Bernard et al., 1992) however it is also thought to have a key role in the general stress response (Roozendal et al., 1997, Bohus et al., 1996) affecting behavioural and autonomic responses during fear processing (LeDoux et al 1988). Activation is frequently observed during stimuli perceived to be unpleasant or induce fear (Morris et al., 1999, Lane et al., 1997, Buchel et al 1998, Critchley et al., 2002). Electrical stimulation of the amygdala has also been shown to evoke feelings of fear and anxiety (Cendes et al., 1994)

Amygdala activation therefore would be expected during pain processing, particularly when perceived as extremely unpleasant and/or threatening. However conversely a number of studies have reported a decrease in activation observed during painful stimulation (Derbyshire et al 1997, Becerra et al 1999, 2001, Petrovic et al 1999, 2004). It has been suggested that the observed deactivation may reflect a cognitive strategy of attenuation by the amygdala of the perceived distress associated with an aversive stimuli (Petrovic 1999).

2.5.1.6 Cerebellum

The cerebellum, which sits at the base of the brain has three anatomically distinguishable lobes; the flocculonodular lobe, the anterior lobe (rostral to the primary fissure), and the posterior lobe (dorsal to the primary fissure). The anterior and posterior lobes are further divided into a midline cerebellar vermis and two cerebellar hemispheres. Neurons in the cerebellar cortex are classified into granular, stellate, basket, Golgi, and Purkinje cells, the most abundant type being the granule cell (Goldowitz and Hamre, 1998).

There remains some controversy in the literature regarding the role of the basal ganglia and cerebellum in pain studies. Not only is activation consistently reported across imaging studies utilising a range of painful stimuli in healthy volunteers (Casey et al., 1996, Svensson et al., 1997, Iadorola et al 1998, Coghill et al., 1999, 2001), and patients (Derbyshire et al., 2002, Becerra et al., 2006, Schweinhardt et al 2006), activation has also been reported during imagined pain (Ogino et al 2006), perception of pain in others (Singer 2004), empathy (Jackson et al 2005, Moriguchi et al 2007) and anticipation of pain (Ploghaus et al 1999).

Despite the cerebellum being one of the frequently reported areas of activation during pain imaging studies (Borsook et al., 2007), such activation is often initially attributed to representing either an actual or planned motor response to the induced pain and in early imaging studies received little attention other than to report activation. Activation during a painful stimulus was demonstrated in the caudal parts of ipsilateral hemispheric lobuli VI and Crus I/II by Helmchen et al., (2003). This area partly coincides with Larsells hemispheric lobuli VII, previously reported to be activated during imagined but not actual hand movement (Lotze et al., 1999), suggesting this activation may reflect motor intention. Furthermore Staud et al., (2007) investigating temporal summation during C-fibre stimulation also found increased activation in the cerebellum correlated with premotor activation. Jueptner (2001) proposes, from movement studies that the cerebellum is specifically concerned with feedback and monitoring of any actual movement taking place whilst the basal ganglia have a greater role in actual or planned

movement. Bingel et al., (2000) and others however now argue that the cerebellum may have a role in nociception that is not motor related.

Helmchen et al., (2003) compared activation patterns during both noxious and innocuous heat stimuli. They suggest that activation in the anterior vermis (lobules II-V) and both cerebellar hemispheres (III-VI) during noxious heat indicates this activation does not reflect pure sensory processing. Additionally the degree of activation increased with pain severity suggesting involvement not only in nociceptive processing but also pain perception.

Projections from the ventral dentate nucleus of the cerebellum to the dorsal prefrontal cortex in primates has been demonstrated (Middleton and Strick 2001) providing evidence for the potential role of the cerebellum in cognitive function.

More recently in humans, Allen et al., (2005) found that changes in the MR signal in the dentate nucleus correlated with signal fluctuations in cerebellar, thalamic, limbic, striatal, and cerebrocortical regions. Correlation was particularly apparent in dorsolateral prefrontal cortex. These findings provide further evidence of a functional relationship between the cerebellum and other brain regions involved in higher cognitive functions.

Whether cerebellar activation seen during pain studies is related to somatosensory, nociceptive, pain perception or motor processing remains unclear.

2.5.1.7 Basal ganglia

The basal ganglia comprises; caudate nucleus, putamen, nucleus accumbens, globus pallidus, substantia nigra and the subthalamic nucleus.
Animal studies show that the basal ganglia receive noxious and non- noxious somatosensory information (Schneider and Lidsky 1981, Chudler et al., 1993) although the functional significance of the nociceptive input is unclear. Activation of the basal ganglia during pain studies, in common with the cerebellum, whilst frequently reported has been attributed to motor planning or intent.

Work by Bingel et al., (2004) suggests that the putamen encodes for laterality of noxious stimuli in a study utilising thulium-YAG laser evoked pain, thereby removing any confounding tactile stimuli. The authors reported somatotopic organisation of nociceptive information in the contra-lateral putamen. Interestingly although activation of the putamen was bilateral, somatotopy was only present contralaterally.

2.5.2 Brain imaging of muscle pain

Imaging studies investigating muscle pain are sparse; those published are limited and inconclusive. Although unclear from these studies if there are variations between the pain matrix for skin and muscle (overlap has been demonstrated), it is likely that there are discrete differences (Niddam et al., 2002). Nonetheless the evidence does suggest that there may indeed be differences in cortical processing of muscle and cutaneous pain. There were until recently only two reports in the literature of the intramuscular hypertonic saline technique being utilised in brain imaging studies.

Svensson et al., (1997) in a PET study investigating forebrain responses in a group of 11 healthy volunteers report the differences between laser evoked cutaneous pain and electrically evoked muscle pain. The authors found no reliable statistical difference in cerebral activation between the two forms of noxious stimulation however report that the ipsilateral premotor cortex, the contralateral prefrontal cortex and SII tended to be more activated in the noxious cutaneous condition whereas the ACC was more responsive to intramuscular pain. The study however has a number of limitations including the fact that only very low levels of pain were evoked; it has been demonstrated that an increasing number of cortical and subcortical areas are activated with increased levels of pain (Derbyshire et al., 1997).

The particular weaknesses of the use of electrical stimulus to induce muscle pain have been covered earlier in this chapter however it is worth noting again that electrical stimulation is a non specific stimulus therefore afferent input can be

assumed to be different between HS induced and electrically induced muscle pain (Korotkov et al., 2002). It is not possible to conclude whether the reported differences between muscle and cutaneous pain in this study therefore can be ascribed to tissue type, stimulation type or indeed a combination of both.

Niddam et al., (2002) also used an electrical stimulus in an fMRI study to investigate neural correlates of muscle pain. During the painful stimulus (in comparison with non-painful stimulus), in addition to areas reported previously by Svensson et al., (1997), activity was reported in areas of both hemispheres including SMA, SMG, thalamus, claustrum, STG posterior cingulate and posterior insula.

The authors conclude that there was substantial overlap between central pain matrices for acute muscle pain and that previously documented for cutaneous pain however these authors did not themselves undertake a direct comparison of muscle and cutaneous pain. Again therefore caution should be used when ascribing differences to tissue type when a number of other factors may be implicated.

Korotkov and colleagues (2002), also utilising PET investigated the changes in regional cerebral blood flow following hypertonic saline induced muscle pain. They report a smaller number of areas than reported previously in pain studies and suggest that this may be due to the low intensity (NRS = 3.5) and duration of pain induced. As Niddam (2002), this group again failed to undertake a direct comparison with cutaneous pain. Of interest however is the report of activity in the putamen, part of the basal ganglia and considered to be an important element of the motor control system.

A more recent PET study (Kupers et al, 2004) reports on HS induced masseter muscle pain. Activation during muscle pain was reported in the ACC (mid-cingulate and rostral perigenual), anterior insula, cerebellum and inferior prefrontal cortex. Interestingly the authors report a decrease in rCBF in the amygdala, ventromedial prefrontal cortex and subgenual cingulate cortex.

Previous studies have reported an increase in amygdala activation during phasic pain and a decrease during tonic pain, the authors suggesting this may reflect a coping mechanism to reduce the unpleasantness of the ongoing pain.

Mechanical hyperaesthesia, a feature of experimental muscle pain, was also included in this study. Whilst muscle pain alone was not associated with any thalamic activation, during mechanical stimulation plus muscle pain posterior thalamus and anterior subgenual cingulate activation was observed which the authors attribute to central sensitisation.

The authors conclude that cerebral processing of jaw muscle pain and cutaneous pain may be different however this hypothesis needs to be tested further and acknowledge that they did not in fact undertake a direct comparison.

Henderson et al., (2006), utilised fMRI in a study investigating 15 healthy volunteers during both HS induced muscle and cutaneous pain; the first group to undertake this direct comparison. Subjects received two single HS bolus injections, one into the right tibialis anterior muscle and one subcutaneously to the overlying skin during two separate scanning runs. Some psychophysical data was collected online however this consisted of a buzzer which was pressed by the subject at onset of the pain, just after the peak intensity had been reached and again when the pain had ceased; A modified Borg scale was used to rate pain intensity however it is unclear whether this data was gathered during or after scanning. Time course of the pain were plotted post scan. Sensory words from the Short Form McGill pain questionnaire were also read out loud to the subjects however again it is unclear whether this was peri or post scan.

Despite employing only a single injection into each tissue type the authors report significant signal change during both conditions in anterior and posterior mid cingulate cortex, posterior insula and SI and MI and during muscle pain only in ipsilateral anterior insula and perigenual cingulate cortex; the latter being a decrease in signal.

Changes within SI were localised to the dorsal aspect of the paracentral lobule during superficial pain whilst during deep pain this signal intensity increase extended to the entire dorso-ventral extent of the paracentral lobule. MI activation was also more extensive during deep pain, extending to the paracentral lobule. The authors attribute this difference to the greater area of pain referral reported during deep compared to superficial pain, reflecting the sensory discriminative role of SI.

The findings of ipsilateral insula activation during muscle but not cutaneous pain is somewhat surprising; the insula as previously discussed being the region of the brain most frequently reported as being activated in previous imaging studies employing acute experimental cutaneous pain.

However this group of investigators have also published further studies employing the HS model reporting on gender differences between muscle and cutaneous pain (Henderson et al., 2008), somatotopy for muscle and cutaneous pain in the insula (Henderson et al., 2007) and associated referred pain (Macefield et al., 2007).

2.5.3 Functional divisions within the 'pain matrix'

The concept of functionally separate medial and lateral components of the human brain system, based on cadaver work and also neurosurgical observation was proposed prior to the availability of modern neuroimaging techniques (Bowsher, 1957; Albe-Fessard et al., 1985). Advances in neuroimaging techniques have allowed the theory of anatomical and functionally separate pathways to be further developed.

The lateral pain system consists of spinothalamic tract neurons projecting to the ventrobasal nucleus of the thalamus, which in turn projects to the primary and secondary somatosensory cortex and the parietal operculum. This so called lateral system is a somatotopically organised, fast, more direct route with fewer synapses than the medial system and is considered to subserve the sensory-discriminative

components of painful experience which include location of the pain, intensity, type and duration.

The medial pain system is slower (in comparison to the lateral system), multi-synaptic, non-somatotopic and comprises the medial thalamic nuclei, anterior cingulate cortex (ACC), prefrontal cortex and insula. The medial pain system consists primarily of spinothalamic tract neurons that project to the intralaminar and medial thalamic nuclei, which subsequently project to the anterior cingulate cortex (ACC), amygdala, hippocampus, and hypothalamus (Sewards and Sewards, 2002). The medial system is predominantly concerned with motivational-affective and cognitive-evaluative aspects of pain processing, memory for pain, and autonomic-neuroendocrine responses (Treede 1999).

Division of function has been demonstrated during functional imaging studies utilising a variety of methods – mainly manipulation of attention. Destruction of somatosensory cortex greatly impairs painful stimulus localisation without altering pain affect; a possible explanation for this being that the medial pain system including cingulate is still intact (Ploner et al., 1999). However in studies utilising varying pain intensities Coghill et al., (1999) and Derbyshire et al., (1997) demonstrated pain intensity coding throughout the pain matrix i.e. it is not limited to just the lateral pain system.

Furthermore the insula receives projections from the posterior part of ventromedial nucleus (VMpo) and as mentioned above somatotopy has been demonstrated in the posterior insula (Ostrowsky et al., 2002, Brooks et al., 2006) suggesting that the insula also has a sensory discriminative role. Chen et al 2007 suggests that this may place the insula in an intermediate position between the lateral and medial pain systems

Although the concept of a functionally distinct medial and lateral pain pathway may be an oversimplification it provides a theory for hypothesis and discussion.

2.5.4 Cognitive and affective effects on pain processing.

One of the difficulties and indeed criticisms in interpreting functional imaging data is the knowledge that there are many factors that influence brain activity rather than just the stimulus itself. Differences reported in activation patterns across a number of studies may be due to methodological considerations, in particular lack of control. Various authors have sought to manipulate a number of attentional and motivational factors to investigate these further.

2.5.4.1 Attention and distraction

Brooks et al., (2002) demonstrated a shift from anterior to posterior insula activation during painful contact heat when attention was distracted away from the stimulus suggesting that distraction attenuates the affective component of the painful stimulus whilst attention enhances it.

Kulkarni et al (2005) elegantly investigated the effect of manipulating subjects to attend to either the localisation or unpleasantness of a painful stimulus and demonstrated a clear difference in functionality of the medial and lateral pain systems. During localisation tasks activation in SI and inferior parietal cortices were reported whereas activation in perigenual ACC, orbitofrontal cortex (OFC), frontal pole, amygdala, posterior insula, MI and hypothalamus was more prominent during the attention to unpleasantness.

Ohara et al., (2004) reported on laser evoked potentials measured directly from a patient with a subdural grid implanted for the treatment of epilepsy and found when the subject attended to the painful stimulus LEP peaks were enhanced in SI, parasylvian and medial frontal cortices when compared to a distraction task.

2.5.4.2 Anticipation

During pain imaging studies subjects may be shown a visual cue indicating the beginning of a painful stimulus or during block designed trials they may learn when a stimulus is due leading to subjects anticipating the onset of pain. Behavioural studies have demonstrated that uncertainty about impending noxious stimulation can both modify the perceived unpleasantness of the stimulus and decrease pain tolerance (Staub et al., 1971). Several authors have therefore investigated the effects of anticipation by manipulating the context of stimuli delivery.

Anticipation of either innocuous touch or painful electrical stimuli to the finger resulted in a decrease in rCBF in SI somatosensory area representing the face i.e. areas of SI located out with that associated with the body location about to be stimulated (Drevets et al., 1995). This would indicate that anticipation not only enhances activation in appropriate regions but may generate a generalised suppression of background activity in the neighbouring region. Porro et al., (2002) report similar findings of increased activation in foot representation area of contralateral (SI) during anticipation of a painful stimulus to the foot. The authors point to this as providing evidence for top-down modulating cortical systems involved in sensory and affective components of pain which may be triggered by anticipation even in the absence of actual noxious input.

Decreased amygdala activation was found when subjects anticipated a prolonged duration of painful stimulation (i.e. more aversive) when compared to a shorter duration despite intensity of pain being matched (Petrovic et al, 2004). The authors suggest this deactivation may reflect a cognitive mechanism that exists to reduce subjective distress experienced whilst in an experimental "no-escape" situation. Anticipation was also associated with an increase in activation in the rostral ACC.

Ploghaus et al., (1999) manipulated subjects' expectations prior to them receiving either a noxious or innocuous heat stimulus. During anticipation anterior medial frontal cortex, anterior insula and posterior cerebellum activation was observed whilst the actual pain stimulus activated caudal ACC, mid insula and anterior

cerebellum. The authors sensibly advise caution when interpreting pain imaging data whereby anticipation has not been controlled for.

In summary, pain is a complex, multi-dimensional experience that comprises sensory-discriminative, affective, motivational and evaluative components. Multiple cortical and sub-cortical areas of the human brain are involved in the recognition, experience and modulation of pain. These areas include primary and secondary somatosensory cortices, thalamus, insula, prefrontal cortex, cerebellum, basal ganglia. Functionally the pain matrix may be considered as a medial and a lateral pathway with separate associated functions. The lateral pathway is primarily associated with sensory-discriminative components of the pain sensation whilst the medial slower pathway is typically associated with affective and evaluative components. Evidence from brain imaging studies to date generally support the existence of functionally separate medial and lateral pain pathways although it should be recognised that this may be an oversimplification.

2.6 Imaging studies of clinical pain

Investigations utilising brain imaging of pain has been dominated by the use of acute experimental pain models (Kupers and Kehlet 2006). Whilst study paradigms have evolved and a number of clever manipulations by investigators have produced results, which have furthered our understanding of acute physiological pain processing; in contrast, investigations involving brain imaging in patients with chronic pain are scarce.

Brain activation patterns observed during acute, physiological pain processing may not reflect that which happens in chronic pain conditions. It has been suggested that a fundamental approach to understanding the pain experience in clinical pain is to establish whether patients display altered cerebral representations of painful stimuli; if so then what is the role of peripheral and central factors in generating this altered representation (Schweinhardt and Tracey (2007).

Increasingly researchers' have turned their attention to these patient populations; assembling a homogenous patient cohort, particularly in respect to matching symptom profile, duration of disease, medication history, age distribution, aetiology and psychological profile is however often problematic (May, 2007). It is perhaps therefore unsurprising that a recent review of clinical pain studies (Kupers and Kehlet 2006) suggests that the results are 'highly incongruent'.

2.6.1 Studies investigating experimental pain in clinical patient populations

On account of the difficulties in reliably inducing / reproducing clinical pain and differentiating between evoked and spontaneous pain within a scanning environment, early brain imaging studies involving chronic pain conditions typically investigated brain activation patterns in patients to an experimental pain stimulus, typically employing contact heat or mechanical pressure as the painful stimulus and usually in an anatomically distinct body area from the reported clinical symptoms.

Jones and Derbyshire (1997) investigated six patients diagnosed with Rheumatoid Arthritis, a chronic inflammatory pain condition; undertaking PET scans during heat pain. In comparison to age and sex matched controls, the patient group showed "remarkably damped cortical and subcortical responses" to a nociceptive input. Areas showing reduction included prefrontal and anterior cingulate areas.

The same group later undertook a similar study in another patient group; patients with acute, unilateral surgical pain, again applying heat pain to the contralateral hand during PET and comparing the responses with the control and RA group (Derbyshire et al., 1999). The surgical patients demonstrated significantly increased regional cerebral blood flow in the ipsilateral prefrontal cortex (BA 44) during heat pain in addition to contralateral increases in the putamen and bilateral increases in the insular cortex. In comparison to the control and RA group, significantly reduced responses in the anterior cingulate (BA 24), pre-frontal medial, and orbito-frontal (BA 9/10/32/47) cortices were shown. They suggest the reduced frontal and anterior cingulate responses to the experimental heat pain during acute inflammatory pain (left jaw) indicates cortical modulation of

nociceptive processing that may be related to non-somatotopic, bilateral, nociceptive inputs to these areas. However it is difficult to extrapolate the results due to the small numbers in each group (N = 6) and the significant difference in age between the surgical and other groups.

Derbyshire et al (2002) compared the activation of a number of brain regions during application of heat at graded intensities in healthy volunteers compared to in patients with chronic low back pain. Only a few small differences were observed between the groups that the authors did not consider were sufficient to indicate that abnormal nociceptive processing in patient group may be occurring.

Fibromyalgia is a chronic pain condition characterised by reduced pressure pain thresholds over 11 of 18 muscle-tendon sites, in the absence of a clinically demonstrable peripheral nociceptive cause (Wolfe et al., 1990). Gracely and colleagues (2002) investigated cerebral responses to noxious and innocuous mechanical pressure in fibromyalgia patients compared to healthy volunteers.

During a matched pressure design patients reported higher levels of pain intensity and increased activation was observed in parietal, insula and cingulate cortices. However when the pain levels were matched i.e. the patient group had a reduced pressure applied, no differences in the level or area of activation was observed between patients and volunteers suggesting that in fibromyalgia, augmented processing by the CNS of peripheral stimuli occurs. Further evidence to support this conclusion of augmented processing in fibromyalgia comes from a recent review of the literature by Schweinhardt et al (2008).

The same group of investigators later undertook a similar investigation in patients with chronic low back pain (Giesecke et al., 2004) investigating pressure pain at a neutral site – i.e. neither within nor in close proximity to the area of clinical pain. During equal pressure, increased activation was observed in pain-related cortical areas in the LBP group compared to controls; contralateral primary and secondary somatosensory cortices, inferior parietal lobule, cerebellum, and ipsilateral SII. In contrast when pain intensity was matched (lower pressure in patient group

compared to control group) the authors report no significant difference in brain activation patterns between the groups.

Cook et al., (2004) in an fMRI study investigated brain activations to both painful and non-painful heat stimulus in nine female subjects with FM compared to healthy controls. Interestingly during warm stimulation that was *non painful*, FM subjects compared to controls demonstrated significantly greater activity prefrontal, supplemental motor, insular, and anterior cingulate cortices. During painful heat stimulus (intensity matched) only contralateral insula showed an increase in the FM patient group when compared to the controls.

2.6.2 Surrogate models of clinical pain

Surrogate models of chronic pain have attracted interest of some researchers in an attempt to 'bridge the gap' between experimental and clinical pain studies whilst minimising the confound of heterogeneity in clinical populations.
The heat capscaicin model of heat allodynia described previously is one such model employed by Lorenz et al., (2002) in healthy volunteers, demonstrating increased activation in medial thalamus, ventral putamen, pACC and prefrontal cortex in comparison with heat pain of similar intensity.

2.6.3 Investigations of clinical pain exacerbation

Schweinhardt et al., (2006) studied brain responses to exacerbation of allodynia in eight patients with a clinical diagnosis of neuropathic pain, demonstrating activation in the rostral anterior insula (rAI) that correlated with perceived intensity of provoked allodynia. The authors also undertook a review of the literature investigating anterior insula activation in clinical pain studies, demonstrating that anterior insula activation reported was more rostral during clinical pain than experimental pain.

Kulkarni et al., (2006), in a PET study investigated twelve patients with osteoarthritis of the knee, undertook scanning during 3 different pain states: arthritic knee pain, experimental knee pain, and pain-free. Although activation of

the pain matrix was seen during both clinical and experimental pain, arthritic pain demonstrated greater increase in activity in cingulate cortex, thalamus, and amygdala; areas which have been reported pain studies but also involved in the processing of fear, emotions, and in aversive conditioning.

Mayer et al., (2005), in a PET study, reported greater activation of limbic and paralimbic circuits during rectal distension in patients with IBS compared with control subjects or patients with quiescent ulcerative colitis.

Baliki et al., (2006) report on a cleverly designed study investigating brain patterns associated with sustained pain compared to spontaneous exacerbations of pain and thermal pain in an fMRI study of patients with low back pain. They report increased activity in the medial prefrontal cortex, rostral anterior cingulate and posterior parietal cortex that was strongly related to intensity of the ongoing pain. In contrast to this was during spontaneous exacerbations of LBP the authors report activation in brain areas previously reported during acute experimental pain.

The authors suggest that their findings of positive correlation between mPFC activation and intensity of spontaneous pain in comparison to the similar relationship between insula activation and pain intensity during thermal stimulation indicates that sustained pain involves specific spatiotemporal neuronal mechanisms that are distinct from those observed for acute experimental pain.

2.6.4 Post therapeutic intervention studies

In an early PET study Di Piero et al., (1991) investigated 5 patients with unilateral chronic pain due to cancer before and after percutaneous cervical cordotomy; an intervention which has been shown to effectively reduce unilateral neuropathic pain. They report a decrease in blood flow of the contralateral (to side of pain) thalamus pre cordotomy that was normalised post successful cordotomy. They also note that they observed no significant changes in the prefrontal or primary somatosensory cortex.

Hsieh et al., (1995), also in a PET study investigated the neural substrates of the perception of chronic neuropathic pain in eight patients with painful peripheral neuropathy in the lower limb before and after a successful regional nerve block lidocaine. The ongoing neuropathic pain resulted in activation of bilateral anterior insula, posterior parietal, lateral prefrontal and PCC and right ACC Brodmann area (BA) 24, regardless of the side of PMN. A reduction in rCBF was noted in the contralateral posterior thalamus but no significant change of rCBF was detected in the somatosensory areas, i.e., SI and SII.

2.6.5 Structural brain changes and pain

Voxel-based morphometry investigations have revealed subtle, regionally specific changes in grey matter across subjects in a number of chronic pain conditions.
May et al., (1999), in a study comparing patients with a history of cluster headache to age matched controls found in the patient group *higher* grey-matter density in the hypothalamus ipsilateral to the side of the cluster headache attacks. The area of increased grey-matter density overlapped with that where increased rCBF during a cluster attack was also found; the authors suggesting parallel structural and functional changes.

Patients with chronic back pain, again compared with a matched control group have been reported as having a 5–11% *reduction* in global cortical grey-matter density; the decrease correlating with chronicity of pain. (Apkarian et al., 2004). More specifically, regional grey matter was also reduced bilaterally in prefrontal cortex and right thalamus.

Two further studies investigated CLBP patients; Schmidt-Wilcke et al., (2006) in a study of 18 patients compared to age matched controls report a significant decrease of gray matter in the brainstem and somatosensory cortex; the change in gray matter showing a negative correlation with degree of unpleasantness i.e. the higher the degree of reported unpleasantness the lower the gray matter density. Conversely they also report a significant *increase* in gray matter bilaterally in the basal ganglia and the left thalamus.

Buckalew et al., (2008) subsequently investigated structural brain differences in older adults with chronic low back pain (CLBP) when compared with age matched pain-free controls; demonstrating significantly decreased gray matter volume in the posterior parietal cortex and middle cingulate white matter volume of the left hemisphere in CLBP patients. CLBP participants also had impaired attention and mental flexibility. However again the numbers in this study were limited (N = 8 in each group).

Other investigators have also reported morphometric changes in chronic tension-type headache (Schmidt-Wilcke et al., 2005) and phantom limb pain (Draganski et al., 2006). N-acetyl aspartate, found localised within neurons, involved in synaptic processes and considered as a neuronal and axonal marker was found to be depleted in the prefrontal cortex, specifically the DLPFC, in patients with chronic low back pain (Grachev et al., 2000).

However, it remains unclear what the relationship of these changes are to pain; similar reductions in grey-matter density have been reported in non pain studies including in bipolar depression (Lyoo et al., 2004) and patients with chronic fatigue syndrome (de Lange et al., 2004). The adult human brain has also been shown to be capable of changes in structure in response to environmental demands (Draganski et al., 2004) therefore a key question with regards to the findings of cortical morphological changes remains; are these a consequence, contributing factor in development or indeed simply an epiphenomenon of chronic pain. Longitudinal studies may help answer this question.

2.6.6 Summary of clinical pain studies

Kupers and Kehlet (2006) advocate repeated-measure, prospective-study designs carried out in a homogeneous subset of patients before and after a well-described surgical intervention as a way forward in investigating clinical pain syndromes. They suggest the post-operative pain model has the advantage of pain onset predictability and that patients may be designated low or high risk for development of chronicity on the basis of their response to an acute pain stimulus before surgery. They point out that the fact that both the low and high-risk

patients undergo the same experimental surgical procedure would allow for the study of time by group interactions in the development of chronicity.
The idea that we may be able to 'track' the development of chronicity through a series of brain imaging studies is certainly appealing, however any such study would require careful planning and significant resource to ensure the multi-factorial nature of chronic pain development is captured.

Published clinical pain studies have used patient populations that differ greatly in demographics related to age and sex, cause of pain, duration of pain, levels of ongoing spontaneous pain and psychological profile making comparisons difficult.

However taken together, a body of evidence exists that in patients with a variety of chronic pain syndromes, when compared to healthy volunteers, neuroplastic changes and alterations in brain processes occur. These differences include, somatotopic re-organisation, augmented (or suppressed) processing of both innocuous and noxious stimuli and morphological changes.

The degree to which these factors are a consequence, contributing factor in development or indeed simply an epiphenomenon of chronic pain remains uncertain.

Author	Tissue	Pain stimulus	vas	Side of activation	MI	SI	SII	ACC	PCC	AI	PI	Th	PFC	PMC	Cer	BG	pre	Brain stem
Svensson et al 1997	M	electrical	7.5	cont	√	√	√			√	√	√	√					
				ipsi										√	√			
	C	CO2 laser	7.5	cont	√	√	√			√	√	√	√					
				ipsi										√	√			
Niddam et al 2002	M	electrical	?	cont		√	√	√	√	√	√	√						
				ipsi	√		√		√		√	√						
	C	N/A																
Korotkov et al 2002	M	HS	3.5							√	√							
	C	N/A																
Thunberg et al 2004	M																	
	e	HS	5	cont														
				ipsi						√						√		
	I		4	cont									↑MPFC					
				ipsi				↓		↓								
	C	N/A																
Kupers et al 2004	M	HS	7	cont				√			√				√	√	√	√
				ipsi	√					√	√		√		√			
	C	N/A																
Henderson et al 2006	M	HS	5	cont	√	√		√	√		√							
				ipsi				*	√	√								
	C	HS	6	cont		√		√	√		√							
				ipsi				√	√									
Henderson et al 2007	M	HS	5	cont	√	√		√	√		√				√			
				ipsi				*	√	√			↓ DLPFC		**			
	C	HS	6	cont		√		√	√		√				√			
				ipsi				√	√	√			↓ DLPFC		**			
Macefield et al 2007	M	HS	5	cont	√	√		√	√		√							
				ipsi				*	√	√			↓ DLPFC					
	C	HS	6	cont		√		√	√		√							
				ipsi				*	√				↓ DLPFC					

Table 2.1 Summary of activation reported in imaging studies utilising experimental muscle pain. M - muscle, C – cutaneous, MI – primary motor cortex), SI – primary somatosensory cortex, SII-secondary somatosensory cortex, ACC – anterior cingulate cortex, PCC – posterior cingulate cortex, AI – anterior insula, PI- posterior insula, Th- thalamus, PFC – prefrontal cortex, PMC – premotor cortex, CER – cerebellum, pre –precuneus BG – basal ganglia * decrease in pACC signal ** decrease in cerebellum signal

2. 7 Summary of chapter

Whilst several methods for inducing experimental pain exist, each with relative advantages and weaknesses, the key requirement for the planned study was a method of induction that may be applied to selectively induce pain in the skin and muscle in a repetitive and safe manner, employing a methodology applicable to both tissue types and that is compatible within an MRI environment.

It was concluded therefore that injection of hypertonic saline as the method of induction of pain offered the best balance.

By far the greatest majority of the pain imaging literature to date has relied on acute experimental cutaneous pain induced in healthy volunteers. The small number of studies utilising muscle pain, whilst limited are sufficient to suggest that pursuing the brain imaging of muscle pain models may be useful in particular in relation to perhaps serving as a more reflective model of clinical pain. This will be further explored in chapter 6.

Increasingly, researchers have become aware that results of brain imaging studies employing experimental pain models may not reflect brain processes involved in clinical pain conditions.

Studies involving clinical patients are increasing apace; evidence suggests that altered processing does indeed occur in clinical pain. Cause and effect relationships however have yet to be established.

Chapter 3

Psychophysical properties of superficial and deep tissue pain

Introduction

Chapter three presents the methodology and results of Experiment One; a laboratory based investigation of the psychophysical properties of hypertonic saline induced pain in muscle (MS), intradermal (ID) and subcutaneous (SC) tissues.

The key aims of experiment one were (i) determine whether the psychophysical properties of deep and superficial pain differ when pain of similar intensity is induced using the same method of noxious stimulus and (ii) to investigate the applicability and reliability of the hypertonic saline model to consider its use in the planned fMRI studies.

3.1 Background

Pain, in common with any form of somatic sensation, is typically described in terms of sensory, cognitive and affective aspects. Sensory aspects generally include location, magnitude and spatiotemporal properties. Affective components include the hedonic aspect – i.e. the degree of unpleasantness of the sensation and a motivational aspect – producing the motivation to limit the experience of the noxious stimulus (Sewards et al 2002). It has been argued that the hedonic and sensory aspects of somatic sensation are represented and processed in parallel but distinct pathways throughout the nervous system and may reflect differences in psychophysical properties of cutaneous and muscle pain.

Sensory and affective components of pain evoked by different modalities have been shown to differ (Rainville et al., 1992). Therefore in order to undertake a true comparison of pain arising from cutaneous and muscle tissues the same type of stimulus should be employed.

Experimental muscle pain induced by intramuscular injection of hypertonic saline, originally described by Kellgren (1938), and subsequently developed by others (Arendt Nielsen et al., 1996; Graven Nielsen et al., 1997) has become a reliable and valid technique for investigating both peripheral and central mechanisms of muscle pain (Graven-Nielsen et al., 1997). The properties of intradermal hypertonic saline have not been similarly investigated; Witting et al., (2001) suggests this may be due to the volume required intradermally.

The aim of this experiment was to undertake a direct comparison of the psychophysical properties of hypertonic saline induced pain in muscle (MS), intradermal (ID) and subcutaneous (SC) tissues. Compared to previous studies employing different modes of induction this study utilised identical noxious stimulation allowing for an accurate comparison of superficial (ID and SC) and deep tissue (MS) pain.

3.2 Methods

3.2.1 Subjects

Sixteen healthy volunteers (6 male, 10 female) mean age 36 (range 19 - 60) participated in the study. Subjects were recruited via advert to staff of The Walton Centre for Neurology and Neurosurgery and the clinical sciences department of The University of Liverpool which included visiting health care students.

Each subject underwent a semi-structured interview that consisted of past medical history and current symptoms. Subjects were excluded if there was (i) history of a cervical and /or upper limb musculoskeletal condition that had at any stage required investigations or treatment, (ii) history of skin disease (e.g., psoriasis, eczema,), (iii) history of injury of upper arm or neck, (iv) ongoing current musculoskeletal symptoms (e.g., pain, weakness, stiffness), (v) any persistent pain (including chronic daily headaches; however occasional migraine attack were allowed), (v) any systemic disease (except mild hypertension well controlled with medication), (vi) any chronic allergic conditions (e.g., urticaria; however, allergic rhinitis was accepted), (vi) history of a psychiatric or neurological condition, (vii) history or suspicion of current ongoing abuse of alcohol or drugs, (viii) current use of medications with analgesic properties even if used for other purposes, e.g. tricyclic antidepressants or non-steroidal anti-inflammatory drugs, (ix) any ongoing non-drug therapy with analgesic properties (e.g., acupuncture for smoking cessation).

The St Helens & Knowsley local research ethics and The Walton Centre for Neurology and Neurosurgery Research Governance committees approved the study protocol and written informed consent was obtained from all subjects prior to enrolment in the study. Documentation relating to ethics and research governance can be found in Appendix A.

All subjects received a written and verbal explanation of the protocol prior to consenting. All testing was carried out in a temperature and humidity controlled laboratory.

3.2.2 Pain Induction

Selection of hypertonic saline solution strength and volume of injectate was based both on the literature (Graven-Nielsen et al 1997) and also preliminary laboratory testing of five volunteers; the latter in particular guided selection of appropriate volumes. (These volunteers subsequently did not participate in the actual study) The primary considerations when selecting these were the aims to induce pain of (i) sufficient intensity (>4/10 on a VAS) (ii) relatively limited duration (iii) similar intensity in all three tissue types

Both 5% and 6% hypertonic saline solution strengths have been reported in the literature; however, 6% is the most consistently utilised. The 6% saline solution also consistently provoked pain in the 5 test subjects and was therefore selected for this study. Volumes injected into muscle, subcutaneous and intradermal tissues were 0.35ml, 0.25ml and 0.2ml respectively.

Disposable plastic catheters (Vygon, 26 g, 25mm) were inserted in to the posterior aspect of the non-dominant forearm, using an aseptic technique, at three separate sites; the extensor digitorum muscle belly, intradermal and subcutaneous tissue between 5-10 cm distal to the muscle catheter in the skin overlying the extensor digitorum muscle. All three catheters were inserted prior to the subject receiving the first injection and removed after the last injection.

Each site was injected twice with the 6% hypertonic saline at intervals of eight minutes in a single session. The order of the first injection into each site was randomised; however, to reduce the likelihood of accommodation the same order was then kept for the second injection. Subjects were blinded to the site at the moment of injection. As they subsequently were asked to indicate the extent of local and referred pain it was not possible to maintain this blinding. All subjects attended two sessions that were separated by a period of at least seven days.

3.2.3 Pain Assessment

Prior to commencing the study subjects received verbal instructions regarding the psychophysical data that was to be collected and familiarised with equipment and documentation involved.

3.2.3.1 Intensity

Intensity of pain was scored on an in-house designed electronic visual analogue scale (VAS) consisting of a handheld pressure sensitive device that outputs data to a dell laptop. A vertical scale is displayed on the computer screen with fixed end points representing 'no pain' and 'worst possible pain'. Subjects by means of the pressure device move a horizontal bar displayed perpendicular to the vertical line to the point on the scale that represents their pain. The software samples the line once per second (and stores it). Calibration was checked after 3-4 subjects to ensure the scale continued to represent 0-100.

Pain intensity was rated continuously for sixty seconds immediately following each injection; from this data highest pain reported, peak VAS, was determined from the output of the electronic VAS. Repeat intensity scores were taken as single recordings at 120 s and 210s post injection.

All VAS data was plotted and area under the curve (AUC) was calculated as a function of VAS and time for the initial sixty second period (AUC_60s) and for the full three minutes of VAS data collection to give a 'total pain' score (AUC_total). AUC measurements were determined using the mathematics software package Sigmaplot; this integrates under curves using the trapezoidal rule which may be used for equal or unequally spaced x values (SigmaPlot, V10.0, Systat Software, Inc., 2006).

3.2.2.2 Unpleasantness

Unpleasantness of a painful stimulus has been shown to be subjectively distinguishable from intensity of the same stimulus (Rainville et al 1992). Subjects received verbal instructions based on those used by Price et al (1987):

"there are two aspects of pain which we are interested in measuring: the intensity, how strong the pain feels, and the unpleasantness, how unpleasant or disturbing the pain is for you. The distinction between these two aspects of pain might be made clearer if you think of listening to a sound, such as a radio. As the volume of the sound increases, I can ask you how loud it sounds or how unpleasant it is to hear it. The intensity of pain is like loudness; the unpleasantness of pain depends not only on intensity but also on other factors which may affect you. There are scales for measuring each of these two aspects of pain. Although some pain sensations may be equally intense and unpleasant, we would like you to judge the two aspects independently."

This has been shown to be effective in conveying the understanding necessary for subjects to evaluate both sensory and affective qualities of induced pain (Duncan et al., 1988, 1989; Miron et al., 1989).
At sixty seconds post injection subjects rated the unpleasantness of the pain on a visual analogue scale where '0' represented 'not at all unpleasant' and '10' represented 'the most unpleasant sensation imaginable'.

3.2.2.4 Quality

The Short Form (SF) McGill pain questionnaire uses a series of words to evaluate the sensory and affective components of pain, is well validated and has previously been used to reflect these properties in both cutaneous and muscle pain conditions (Klepac et al 1981).

After rating unpleasantness, subjects completed the SF McGill pain questionnaire.

3.2.2.5 Area of pain

Subjects used a fine temporary marker to outline the area of both local and referred pain anywhere in the injected arm. As the non-dominant arm was being tested, the dominant arm was free for marking.

Referred pain was defined as pain after injection in an area separate and distinct from the area immediately surrounding the injection site and subjects were instructed to differentiate between the two.

After confirming the correctness of the indicated area the outline was transferred to transparent acetate and the areas measured with a digital planimeter (Klonk, Quantify One, Denmark)

3.2.2.6 Allodynia

The presence of both punctate (static) and mechanical (dynamic) allodynia was tested for within the area of local pain; this area having been previously indicated by the subject. Where referred pain was present, allodynia was also tested for within that area, again as indicated by the subject.

Punctate (static) allodynia

A Von Frey filament (30g) was placed within the area of pain and held in place for 5 seconds whilst sufficient pressure was applied to cause the filament to bend. Subjects were asked whether this resulted in any increase in pain and if so to rate the resulting pain on a visual analogue scale. Prior to testing within the area of pain, the test was carried out in the mirror area on the opposite limb to confirm that the selected Von Frey filament caused only mechanical pressure not pain in the control area.

Mechanical (dynamic) allodynia

Using a 2cm wide soft brush, five strokes (3-5cm long) were applied within the area of pain. This was repeated 5 times at a frequency of <0.2Hz (to avoid a summation effect). Again, prior to testing the affected side, the test was carried out on the mirror site to allow for subject's to compare the sensations evoked.

Subjects were asked whether this resulted in any increase in pain and if so to rate the resulting pain on a visual analogue scale.

3.2.3 Statistical Analysis

As stated above, three types of injection (ID, MS, SC) were given each on four occasions (two at time one and two at time two) to all participants. In order to examine whether there were any significant differences between each injection type and also to determine any differences between the same type of injection on different occasions, a series of two factor within subject ANOVAs were conducted.

The independent variables were injection type (ID, MS, SC) and time (1, 2, 3 & 4; where 1&2 were the first session and 3&4 the second session). The dependent variables considered in separate analyses were: pain intensity, unpleasantness, time to peak intensity (VAS), AUC_60 and AUC_total.

Mauchly's Test demonstrated that assumptions of sphericity was violated for the interaction term injection*time in the pain intensity analyses (X^2 = 33.6, *df*20, p=.03), therefore Greenhouse Geisser adjusted F values are reported for this effect.

Bonferroni pairwise comparisons were used to determine the differences for significant effects.

Kruskal-Wallis was used to analyse differences in SF McGill data.

SPSS v14 (SPSS Inc., 2006) was used for all analyses.

3.3 Results

3.3.1 Pain intensity: peak visual analogue scale (VAS) score (see appendix B for full SPSS output)

Mean peak VAS score across all injections for ID, SC and MS were 5.5 (2.0), 4.1(2.3) and 5.0 (2.0) respectively (see table 3.1).

The two factor within subjects ANOVA revealed a significant difference between each type of injection ($F(2,30) = 13.20$, $p<.0005$. Partial eta squared = .47) and Bonferroni pairwise comparisons showed these differences to be between ID& SC and SC & MS (injection type 1 & 2, and 2 &3.)

There was no significant interaction between injection type and time ($F(6, 90) = 2.41$, $p>.05$).

3.3.2 Area under the curve; initial 60 second period (AUC_60) (see appendix B for full SPSS output)

Mean area under the curve for the initial 60s period (AUC_60) across all injections for ID, SC and MS were 272.4 (s.d., 15.9), 178.4 (s.d., 15.6) and 182.6 (s.d., 14.6) respectively (see table 3.1).

There was a significant difference in area under the curve between injection types. $F(2, 28) = 23.10$, $p<.0005$. Partial eta squared = .623. There was also a significant difference between injections at each time point $F(3, 42) = 5.89$, $p=.002$. Partial eta squared = .296. The planned Bonferroni comparisons show the significant difference to be between injection type ID & SC and ID & MS but not between SC&MS.

There was no significant interaction between area under the curve and time $F(6, 84) = 1.58$, $p>.05$. Therefore there was no significant difference within injection type across the four different time points

3.3.3 Area under the curve – total time period (AUC_total) or "Total pain" (see appendix B for full SPSS output)

There was a significant difference in AUC_total between each injection type ($F(2,28) = 4.27$, $p<.05$. Partial eta squared =.234).

There was also a significant difference in AUC_total at each time for all injection types $F(3, 42) = 11.29$, $p<.0005$. The planned Bonferroni pairwise comparisons

show that the difference in AUC_total was between injection types ID & SC, not between ID &MS or SC & MS.

The interaction between injection and time was not significant F (2.13) = .58, p>.05. Therefore there was no significant difference within injection type across the four different time points

Table 3.1 Summary of psychophysics data

	N			
	Statistic	Mean	Std. Error	Std. Deviation
Peak intensity				
ID	64	5.5	.25	2.00
SC	64	4.1	.29	2.31
MS	64	5.0	.25	1.98
Unpleasantness				
ID	64	4.6	.29	2.33
SC	64	3.5	.29	2.36
MS	64	4.1	.28	2.27
AUC_60				
ID	60	272.4	15.93	122.93
SC	60	178.4	15.64	121.20
MS	60	181.6	14.61	113.15
AUC_total				
ID	60	636.37	39.28	304.27
SC	60	536.060	44.14	341.94
MS	60	554.67	42.30	327.72
Time to peak VAS				
ID	64	31.73	1.77	14.17
SC	64	40.14	1.75	13.96
MS	64	45.06	1.72	13.743

3.3.3 Area under the curve – total time period (AUC_total) or "Total pain" (see appendix B for full SPSS output)

There was a significant difference in AUC_total between each injection type ($F(2, 28) = 4.27$, $p<.05$. Partial eta squared =.234).

There was also a significant difference in AUC_total at each time for all injection types $F(3, 42) = 11.29$, $p<.0005$. The planned Bonferroni pairwise comparisons show that the difference in AUC_total was between injection types ID & SC, not between ID &MS or SC & MS.

The interaction between injection and time was not significant $F(2.13) = .58$, $p>.05$. Therefore there was no significant difference within injection type across the four different time points

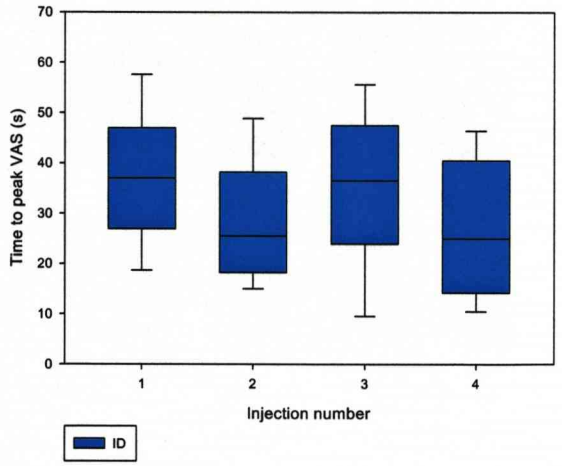
3.3.4 Time to peak VAS (see appendix B for full SPSS output)

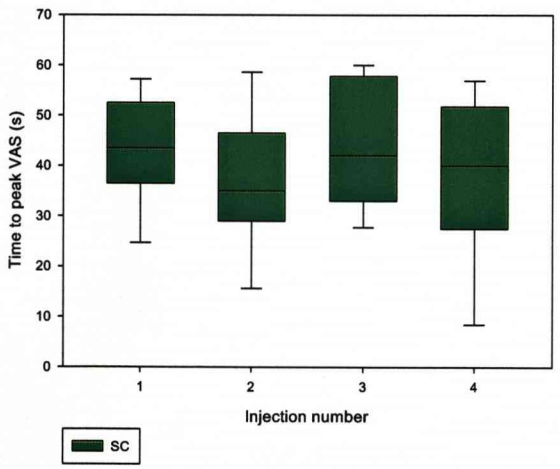
There was a significant difference in time to peak vas for each type of injection ($F(2,30) = 13.85$, $p<.0005$. Partial eta squared = .48.). Here the planned Bonferroni pairwise comparisons show the difference to be between ID & SC and ID & MS ($p<0.05$) with no significant difference between SC and MS ($p>0.05$).

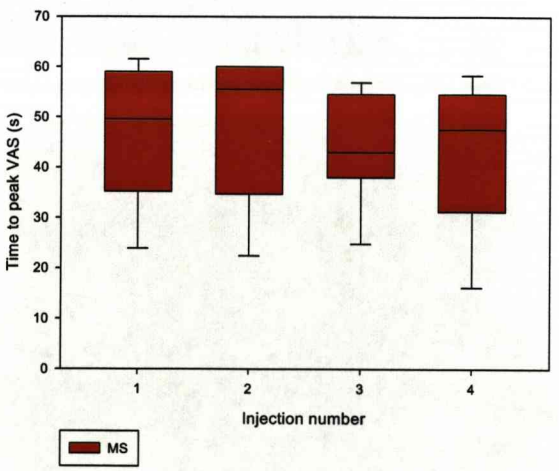
There was no significant effect of time, i.e. no differences between each injection ($F(3, 45) = 2.07$, $p >.05$)

Finally there was no significant interaction between injection and time ($F(6, 90)$ .997, $p>.05$). Again therefore there was no significant difference within the injection type across the four different time points

Figure 3.2. Time to peak VAS intensity at each injection time, arranged according to tissue type.
(Y axis = time to reach peak VAS score in seconds; X axis = injection number: 1 & 2 undertaken during first session, 3 & 4 during second session)







3.3.5 Unpleasantness ratings (see appendix B for full SPSS output)

The two factor within subjects ANOVA revealed a significant difference between each type of injection for unpleasantness ratings ($F(2, 30) = 5.65$, $p<.01$. Partial eta squared = .27)

There was also significant differences between each time ($F(3, 45) = 12.28$, $p<.0005$). Bonferroni pairwise comparisons demonstrate the significant differences to be between injection type ID & SC.

There was no significant injection time interaction ($F(6,90) = 1.76$, $p>.05$). Therefore there was no significant difference within the same type of injection across the four different time points

Unpleasantness was found to correlate with intensity in all three conditions (Pearsons r = .836, p<.000 (ID), r = .820, p<.000 (SC), r = .678, p<.000 (MS) (see table 3.2 and figure 3.1.).

Table 3.2, Correlation of peak intensity VAS and unpleasantness VAS

Tissue	N	Pearson Correlation	Sig. (2-tailed)
ID	64	.836(**)	.000
SC	64	.820(**)	.000
MS	64	.678(**)	.000

** Correlation is significant at the 0.01 level (2-tailed).

Figure 3.3 Mean intensity and unpleasantness scores at each injection time, arranged according to tissue type.
(Y axis = peak VAS score left column , peak unpleasantness right column; X axis = injection number: 1 & 2 undertaken during first session, 3 & 4 during second session)

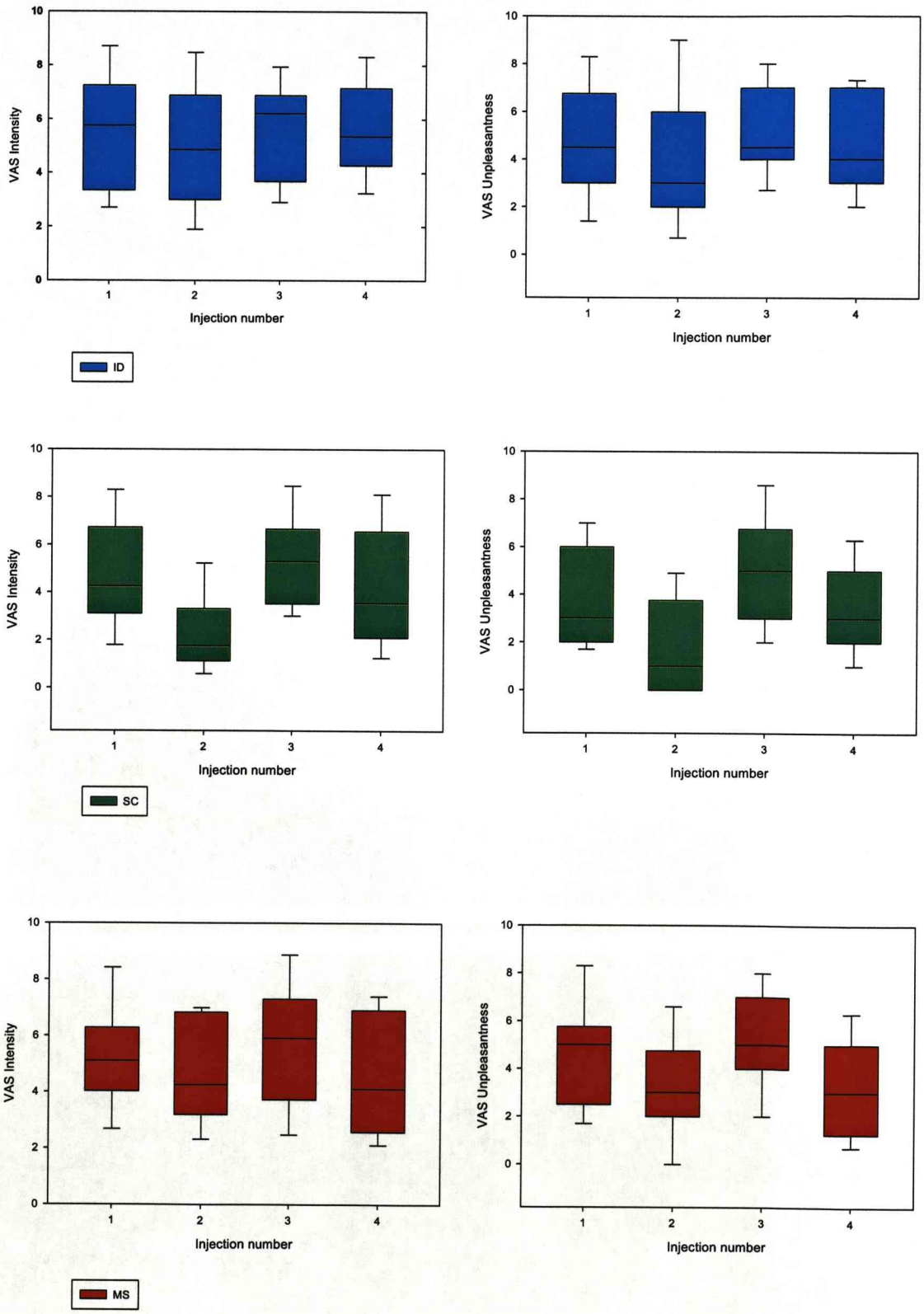


Figure 3.4 Scatter plots of unpleasantness and peak intensity for each tissue type.
(ID – intradermal, SC - subcutaneous, MS - muscle)

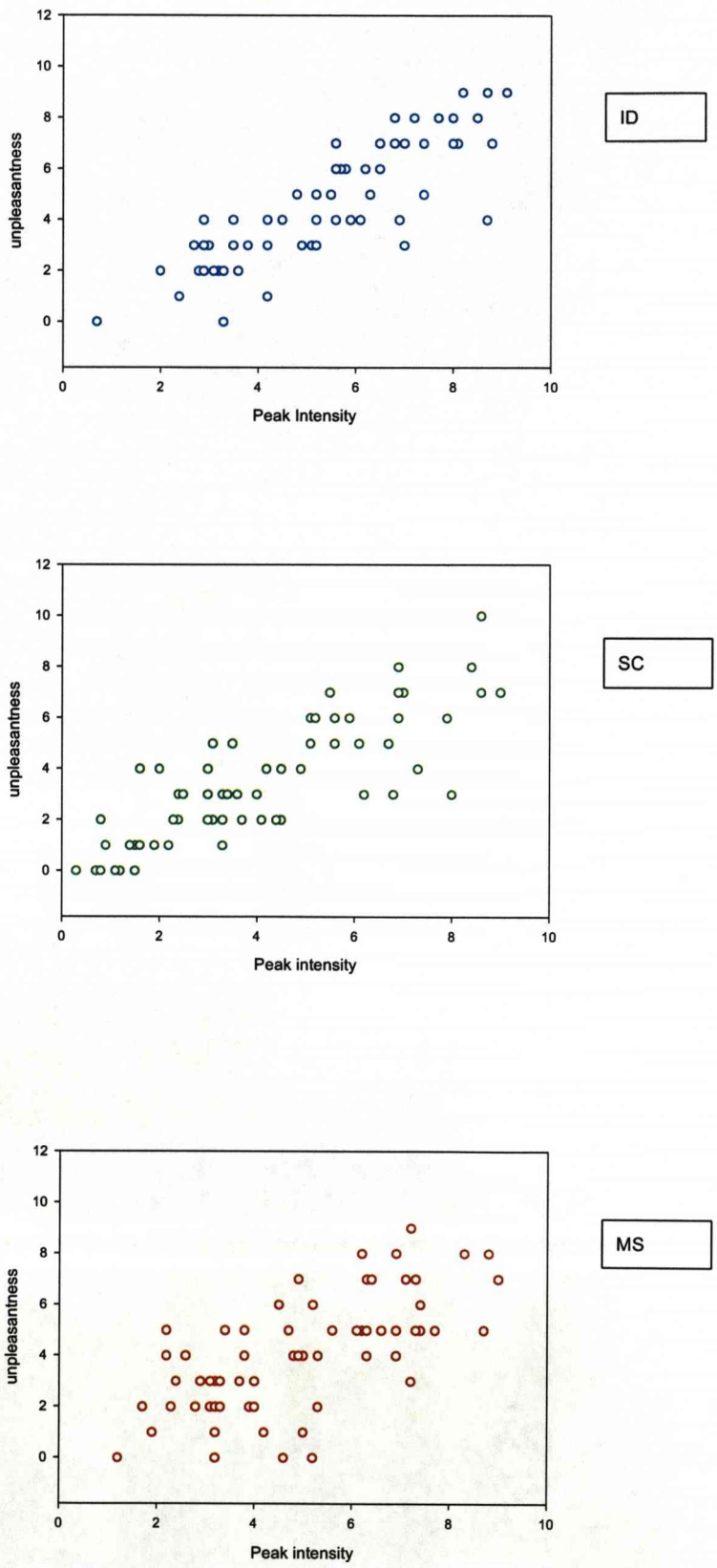
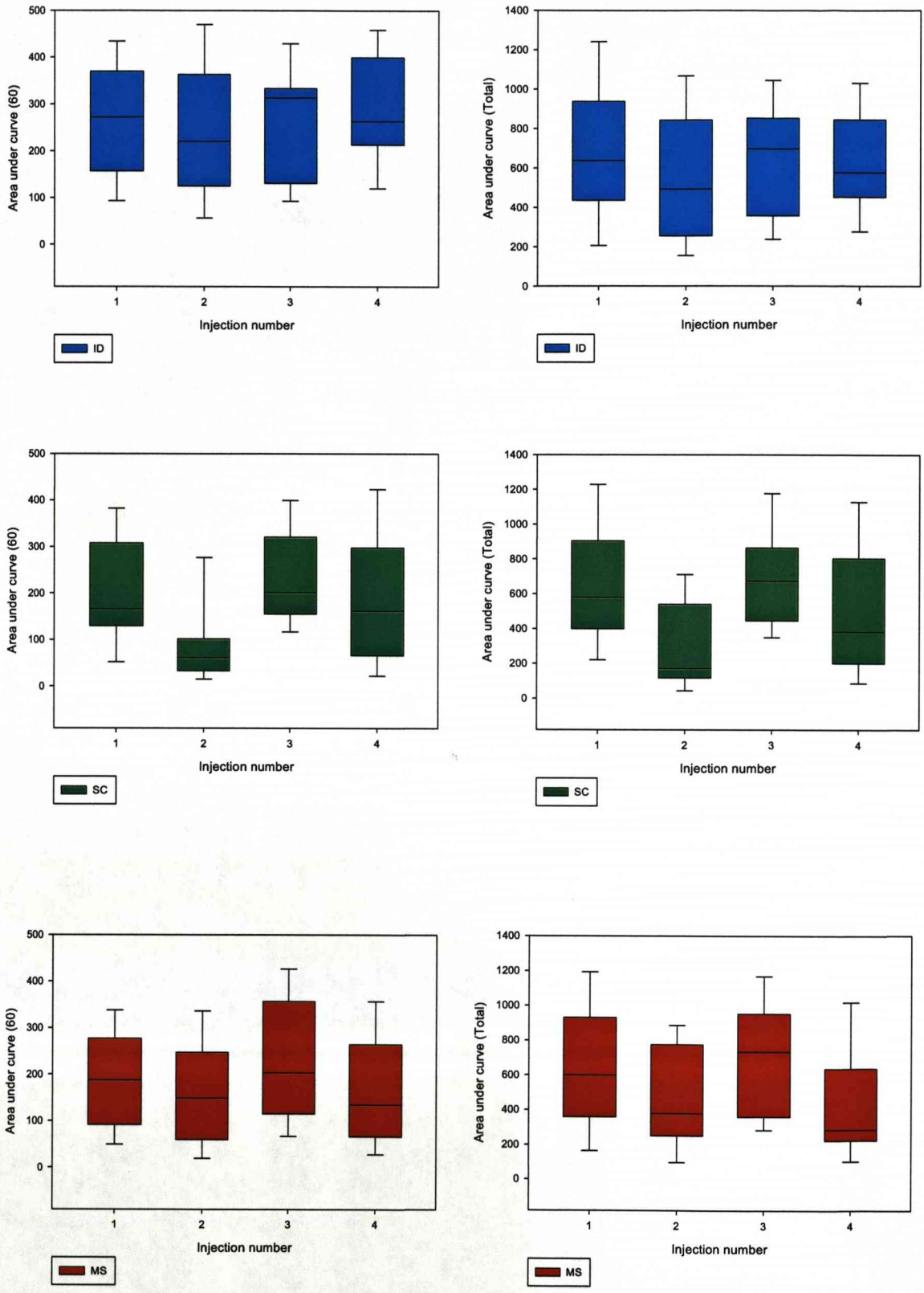


Figure 3.5 Mean area under the curve* for initial 60s (AUC_60) (left column) and total time period (AUC_total) (right column)



* Units of area are arbitrary

3.3.6 Area of local pain (see appendix B for full SPSS output)

The two factor within subjects ANOVA revealed a significant difference between each type of injection ($F(2,30) = 7.98$, $p<.002$. Partial eta squared = .35) and Bonferroni pairwise comparisons showed these differences to be between injection type ID & MS.

There are was also significant differences between each time ($F(3,45) = 3.04$, $p<.05$). Bonferroni pairwise comparisons demonstrate the significant differences to be between times 3 & 4, i.e. within session two.
No significant interaction between injection type and time was found ($F(6, 90) = .75$, $p>.05$).

3.3.7 Referred pain

A separate area of referred pain was reported after 1, 8 and 28 injections for ID, SC & MS pain respectively. In view of the infrequent findings referred pain in both ID and SC conditions no formal statistical analysis was undertaken. Measured area results however are summarised in table 3.3

3.3.8 Allodynia – punctate and brush

Both punctate and brush allodynia, tested in the area of local pain was found infrequently (Punctate; N = 16, 19 & 14 and Brush; N = 14, 8 & 3 for ID, SC and MS respectively). Where present, VAS scores were low (Punctate; vas = 2.1 (sd .85), 1.8 (sd .21) & 1.9 (sd .22) and Brush; VAS = 2.2 (sd 1.2), 2.0 (sd. 1.07) & 3.3 (s.d.1.52) for ID, SC and MS respectively).

In the area of referred pain brush allodynia was found once after one MS injection only (VAS = 3); whilst punctuate allodynia was found three times, after MS injection (one each in three different subjects), (VAS = 1.7, sd 1.15).

In view of the infrequent findings of either punctuate or brush allodynia, in addition to the very low VAS scores reported when present, no formal statistical analysis was undertaken. Results however are summarised in table 3.

Table 3.3 Summary of area of local and referred pain, frequency of referred pain and punctuate and mechanical allodynia (brush) frequency and VAS (where present.

	Frequency [a]	Area		
Site	N	Mean	Std. Error	Std. Deviation
Local pain				
ID	64	31.3	3.07	24.55
SC	64	36.3	3.46	27.64
MS	64	52.2	4.24	33.91
Referred pain				
ID	1	.3	.30	2.40
SC	8	1.8	.64	5.10
MS	28	13.2	3.34	26.79
		VAS score		
	Frequency [b]	Mean	Std. Error	Std. Deviation
Brush allodynia	N			
ID local	14	2.2	.32	1.20
ID referred	0			
SC local	8	2.0	.38	1.07
SC referred	0			
MS local	3	3.3	.88	1.52
MS referred	1	3.0		
Punctate allodynia	N			
ID local	16	2.1	.21	.85
ID referred	0			
SC local	19	1.8	.21	.89
SC referred	0			
MS local	14	1.9	.22	.83
MS referred	3	1.7	.67	1.15
	0			

Frequency [a] - number of times referred pain was reported
Frequency [b] - number of times where a VAS > 1 was reported.

3.3.9 Short form McGill Pain Questionnaire

There was a significant difference in sensory words selected in the ID and MS conditions when compared with the other two conditions.

During ID pain, 'hot/burning', 'stabbing' and 'sharp' were selected more frequently and scored higher ($p<0.05$) than both MS and SC. The word 'shooting' during ID scored higher than MS but not the SC condition.
During the MS condition 'gnawing', 'aching', 'heavy' and 'cramping' were selected more frequently and also rated higher ($p<0.05$) than both the ID and SC conditions.
During the SC condition no descriptor was selected more frequently or scored higher than either ID or MS conditions.

Overall affective descriptors were selected less frequently than sensory words, however when selected this was more frequent in muscle pain (ID 6, SC 9, MS 14). Due to the infrequent selection, no formal analysis was undertaken.
Frequency of selection is summarised in table 3.4.

Table 3.4 Frequency of word selection from the SF McGill pain questionnaire

Sensory	throb	shoot	stab	sharp	cramp	gnaw'g	Hot / burn'g	ache	heavy	tender	Split'g
Injection type											
ID	18	13	29	51	4	2	28	15	2	21	7
SC	19	12	17	31	12	4	18	35	9	15	6
MS	30	2	9	7	31	24	8	32	25	11	3

Affective	Tiring	sickening	fearful	cruel
ID	1	2	1	2
SC	1	5	1	2
MS	4	6	0	4

3.4 Discussion of findings

3.4.1 Intensity of pain

During hypertonic saline induced experimental pain in healthy volunteers into both intradermal and muscle independently, peak VAS scores were of similar magnitude within session and between sessions. Remarkably; peak VAS scores were also very similar irrespective of whether the injection was ID or MS. In contrast, SC injections did not perform as consistently resulting in VAS scores that were less comparable than those reported after ID or MS injections.

Therefore it may be argued that ID and MS hypertonic saline injections can be incorporated into a protocol in which pain of comparable intensity is required between cutaneous pain and deep tissue pain; whereas SC injection would offer a rather less robust model.

3.4.2 Temporal characteristics

Time to peak pain was shorter in the intradermal condition compared to both the muscle and subcutaneous conditions which is consistent with previously reported findings of comparisons between cutaneous and muscle experimental pain (Graven-Nielsen et al., 1997; Witting et al., 2000).

Unsurprisingly therefore area under the curve for the first 60 seconds was also greater after intradermal injection than both subcutaneous and muscle injections which is also consistent with previous reports comparing intradermal and muscle capsaicin injection (Witting et al., 2000).

However total pain (AUC_total) scores showed no significant difference between intradermal and muscle injections; in both conditions this measure was also consistent across all four injection times. In contrast subcutaneous AUC_total was significantly less than intradermal, although not muscle.
These results suggest that although the onset curve for ID is steeper, the offset curve for MS is slightly flattened, therefore 'total pain' reported for the duration

of measurement showed no significant difference between intradermal and muscle injection.

The consistent performance of ID and MS injections in producing comparable amounts of pain over three minutes indicate these models may be useful in study protocols in which pain of moderate intensity but time limited pain is required.

Again, in contrast, SC injections provide a lesser stable model.

3.4.3 Unpleasantness

With regards to intensity of unpleasantness of induced pain, both MS and ID injections produced similar scores with no significant change within or between sessions. A strong positive correlation was found between intensity and unpleasantness scores in both conditions

This is somewhat in contrast to previous reports (Rainville et al., 1992; Svensson et al., 1997a). However, although both of these studies investigated differences between pain arising from deep and superficial tissue, both groups employed different methods of pain induction for the two tissue types.

3.4.4 Extent of pain

Referred pain was a feature of the muscle pain condition but not intradermal. Although a few incidences of referred pain after subcutaneous injection were reported; overall the results suggest that referred pain is a feature arising from introduction of a nociceptive stimulus to deep but not superficial tissue.

Area of local pain was greater in the muscle than the intradermal condition; again this is consistent with the literature with cutaneous pain being described as typically well defined and localised (Staahl et al., 2006) in contrast to the more diffuse, less defined quality of muscle pain (Graven Nielsen et al., 1997).

It should be mentioned however; areas of local and referred pain were measured separately with subjects asked to delineate between the two. A judgement had to be made therefore regarding overlap of the two which may have falsely increased the local pain results and decreased the incidence of referred pain.

3.4.5 Hyperalgesia

Interestingly, incidence of allodynia was low across all three conditions although punctate allodynia was more frequently seen than brush allodynia; where present the VAS scores were also low. Presence of cutaneous allodynia was specifically tested for as Kupers et al., (2004) employed 'mechanical hyperaesthesia as one of their comparators during a PET study investigating neural correlates of muscle pain. Previous studies of sensory changes associated with experimental muscle however are inconsistent; Hockaday and Whitty (1967) reported inconsistency in both the extent and appearance, often delayed, of hyperalgesia. Vecchiet et al., (1988) also report a gradual onset of cutaneous hyperalgesia after painful electrical stimulation of the muscle; conversely Graven Nielsen et al., (1997) observed hypoalgesia to both pain prick and light touch after intramuscular HS infusion. Animal studies also point to differing temporal patterns in the development of hyper / hypoalgesia; after electrical induced referred muscle pain in rats Giamberardino et al., (1988) demonstrated an initial hyperalgesia which changed in time to hypoalgesia.

3.4.5 Qualitative differences

The results indicate that experimental cutaneous and muscle pain, when induced through the same mode of stimulus, can be differentiated through subjective descriptors of quality by completion of the SF McGill Pain Questionnaire. Descriptors selected were predominantly from the sensory as opposed to the affective category of words which is consistent with previous reports (Graven Nielsen et al., 1997; Beattie et al., 2004)).

Although it has been suggested that experimentally induced muscle pain may result in a relatively larger activation of affective mechanisms (Svensson et al., 1997a); this was not reflected by selection of affective words from the SF McGill pain questionnaire.

However when affective words were selected there was a trend for these to be after muscle injection, selection of words from this category was too infrequent to undertake any sensible analysis. Affective word selection therefore did not strongly support the hypothesis that muscle pain would results in a greater selection of these than intradermal and subcutaneous pain.

It is likely that the results reflect both the study population of healthy volunteers as opposed to a patient population particularly those with chronic pain and the experimental situation in that participants were aware that induced pain would be of relatively short duration.

3.5 Summary

In studies in which perceptual differences between cutaneous and deep tissue pain are assessed, the similarity of peak pain and total pain between the two modes of injections (ID and MS) affords a unique opportunity. If the aim is to evaluate neural correlates of the different qualities of pain the subjects feel (as per their responses on SF McGill), there is a very obvious advantage in a model where the likelihood of maintaining pain intensity and unpleasantness levels as standard.

In contrast, reliability of the subcutaneous condition was not found which would suggest caution when investigating certain experimental models which may not stimulate specifically either cutaneous or muscle nociceptors; particularly if the aim of investigation is to explore differences between superficial and deep tissue pain specifically. Comparative studies that evaluate differences between ID and SC may not therefore be reliable. The significant variability seen within the SC pain model excludes it as a useful model to compare with a muscle pain model in further investigations.

The robust ID and MS results also suggest that it may be possible to explore activations in relation to generic pain features, such as unpleasantness. An exploration of perception of unpleasantness with a hypothesis that a certain brain region or circuitry is responsible for generating the percept, irrespective of the mechanism, is much easier if the intensity and total burden of experimental pain can be controlled (e.g., if the hypothesis under testing is, say, that activation in insula co-varies with unpleasantness scores and does so after both ID and MS injections).

The presented results are consistent with the first hypothesis that when subjected to a similar peripheral stimulus, healthy subjects perceive pain arising from skin and muscle tissues as qualitatively different.

The results however did not support the hypothesis that when subjected to a noxious stimulus of the same type into cutaneous and muscle tissues; the resulting intensity matched muscle pain when compared to cutaneous pain will be perceived as more unpleasant.

This provides the foundation for the next hypothesis that neural correlates of each pain will de different. The differences are likely to found in the activation patterns of structures that have been broadly referred to as the 'pain matrix' and provides the rationale for the next investigation.

Based on these findings, ID and MS injections were chosen as the method of provoking pain in subjects undergoing fMRI of the brain which is described in chapter four.

The next two experiments reported in this thesis utilised functional magnetic resonance imaging of the brain.

This chapter therefore begins with an overview of the methodology involved in fMRI acquisition and the analysis principles and procedures undertaken.

Methods specific to the main experiment described in this chapter are then described and psychophysical and fMRI results presented.
The chapter concludes with a brief discussion of the key findings of the experiment presented, with further discussion to follow in chapter 6.

4.1 Functional Magnetic Resonance Imaging

Functional Magnetic Resonance Imaging (fMRI) studies rely on what has been termed the Blood Oxygenation Level Dependent contrast or BOLD response.

Haemoglobin in the blood, when bound to oxygen is diamagnetic whereas deoxygenated haemoglobin is paramagnetic and therefore has a higher magnetic susceptibility (Ogawa et al 1990). In diamagnetic material the magnetic flux is reduced whereas in paramagnetic material the magnetic flux is enhanced or in other words the magnetic field is 'attracted' to it. Hence a change in haemoglobin oxygenation will bring about changes in the local distortion of any magnetic field that is applied to it.

Ogawa et al (1990) demonstrated this in a cat made hypoxic, gradient echo magnetic resonance (MR) images of the animals brain showed signal loss around the blood vessels; the effect being reversed when baseline levels of oxygenation were restored. The deoxygenated blood produced an increase in the blood vessels magnetic susceptibility in relation to the surrounding brain tissue, in turn generating local field gradients and locally decreased tissue T2* in tissue water surrounding the blood vessels. Ogawa further suggested the potential for this phenomenon to be utilised in measuring small changes in the relative blood oxygenation levels that occur with neural activity in the brain. When an area of the brain is active there is a corresponding increase in local blood flow bringing oxygenated blood to the area. The increase in local blood flow far outweighs that which is actually required for local tissue metabolism and leads to a decrease in magnetic susceptibility and therefore an increase in the fMRI(BOLD) signal.

The increase is relatively small (2-5%) and questions have arisen as to whether increase in signal actually reflects neuronal activity. However work by Logothesis et al (2001) has demonstrated that local field potentials (LFPs) correlate with the BOLD signal during a visual stimulation task providing evidence that fMRI (BOLD) contrast does indeed provide a measure of neuronal activity in the brain albeit an indirect measurement.

4.1.1 Temporal resolution of fMRI (BOLD)

When a stimulus is presented to an individual causing a change in brain activity there is an initial small dip in BOLD signal intensity that occurs over the first second following the stimulus presentation. Following this there is a progressive increase in the signal intensity over the next 2-4 seconds that remains at a relatively constant level for the duration of stimulation (Bandettini et al 1997). It is suggested that the initial dip reflects initial deoxygenation of capillary blood due to the high oxygen requirements of local neuronal activity.

This is followed in the next 2-5s by an increase in blood flow which is far in excess of requirements for oxygen therefore increasing the oxyhaemoglobin / deoxyhaemoglobin ratio and hence the signal intensity (Malonek et al 1997). On removal of the stimulus the signal decreases over a few seconds reaching a level that is actually below the initial baseline termed the 'undershoot', after which it then recovers back to baseline over a few seconds. The undershoot is thought to be due to a slowly resolving increase in cerebral blood volume post stimulus (Mathews 2001).

Overall therefore there is an average 4-6 second time lag in the expected response time between the stimulus presentation and the peak of the haemodynamic response measured in the brain.
Variations in the time lag exist between individuals (Aguirre et al., 1998) and also in cortical areas and different tasks (Rajapaske et al., 1998).

4.1.2 Spatial Resolution of the BOLD response

The ability to distinguish change in an image across different spatial resolutions is a particular strength of fMRI; the spatial resolution being given by the voxel size used for functional acquisition. As voxel volume is decreased, although there is an increase in the functional contrast to noise ratio (CNR) there is also a decrease in the signal to noise ratio (SNR) (Macovski, 1996).

The increase in CNR results from the minimisation of partial volume averaging of gray matter (where BOLD signal changes occur), and other tissue e.g. white matter and cerebrospinal fluid, where BOLD effects do not occur. Conversely a reduction in voxel size will also result in a reduction in the overall signal that can be measured from that voxel and hence the likelihood of a reduced signal to noise ratio (Howseman et al., 1999). Therefore it is the vascular nature of the BOLD response that limits the spatial resolution.

4.1.3 Analysis of fMRI data

Signal changes within fMRI data are relatively small and data gathered is inherently noisy therefore to enhance the signal to noise ratio i.e. extract the signal for statistical analysis a number of pre-processing steps are undertaken.
All fMRI analysis described in this experiment utilised tools from the FMRIB FSL toolbox (Smith et al., 2001).

4.1.3.1 Pre-processing; motion correction.

Subject head movement in the scanner can be reduced by fixing with head restraints however inevitably some degree of motion will occur. Movement can result in any signal that is obtained not corresponding to exactly the same voxel throughout the duration of the experiment. The problem can be overcome by aligning all volumes from an experiment with a single volume, which was implemented using FMRIBs linear image registration tool (MCFLIRT, Bannister and Jenkinson 2001, Woods et al 1999).

FLIRT uses a rigid body transformation that takes the assumption that a single voxel in the brain may change position and size but not shape.

4.1.3.2 Spatial filtering ('blurring')

Spatial filtering also known as 'smoothing' is applied for two reasons. Firstly smoothing can enhance the signal to noise ratio by reducing the noise level whilst retaining the underlying BOLD signal. To ensure the signal itself is not lost during

smoothing it is essential that the extent of smoothing is not greater than the size of the activated area.

Secondly in order to meet the assumptions that underlie the statistical theory of Gaussian Random Field Theory that is later applied to the data, spatial smoothing must have occurred.
In the studies reported here a smoothing kernal of 5mm full width half maximum (FWHM) of all fMRI data was applied.

4.1.3.3 Temporal filtering

Temporal filtering is applied to fMRI data in order to remove unwanted components of a time series whilst retaining the signal of interest. Unwanted components may be of high or low frequency and therefore both high-pass and low-pass filtering is applied to each voxels time series.

High pass filtering removes slowly varying unwanted signals in each voxel which may include physiological confounds e.g. cardio- respiratory or non-physiological scanner drift. By removing low frequency signals that are not related to the stimulus and therefore signal of interest apparent noise in the data is reduced in statistical analysis.

During block designed experiments it is of particular importance to ensure that the cut-off period of the filter is not set too low (Smith 2001) otherwise the signal of interest may be lost.

The aim of low pass filtering is to reduce high frequency noise, again without affecting the signal of interest. The risk of removing the signal of interest in experiments that use an event related design where signal of interest may be rapidly changing are particularly high.

FSL implements instead 'Prewhitening' as it is considered more efficient to not carry out low pass filtering and instead 'pre-whiten' the data within statistical analysis (Woolwich et al., 2001).

4.1.4 Statistical Analysis

FEAT implements data modelling that is based on general linear modelling (GLM), otherwise known as multiple regression. The experimental design is described from which a model of explanatory variables is created and then fit to the data. A good fit of the data to the model implies that the data were caused by the stimulus from which the model arose.

The FEAT tool implements a GLM of analysis that is univariate therefore the signal time course from each voxel is separately fitted to the time course of the model.

GLM method used on first-level (time-series) data is known as FILM (FMRIB's Improved Linear Model). FILM uses a robust and accurate nonparametric estimation of time series autocorrelation to prewhiten each voxel's time series; giving improved estimation efficiency compared with methods that do not pre-whiten (Jenkinson 2001).

The GLM may be represented by a simple equation: $\gamma = \beta x + e$
Where γ is the data, x is the model factor, e is the error in the data and β is the parameter estimate or parameter 'weight' i.e. is a scaling factor.

In order to test the significance of a model factor for a given voxel, the PE is divided by the error in the estimate of this PE value, also known as the residual, resulting in a **t** value.
A low PE relative to estimated error is interpreted as the fit being non significant therefore **t** reports how significantly the data is related to a particular explanatory variable i.e. is a good measure of whether we can believe the estimate of the PE value.

Standard transformations to convert a **t** value into a P (probability) or z (gaussianised 't') statistic is then applied producing a statistic map of activation.

Statistical inference or thresholding is then applied to find which parts of the brain were activated at a given level of significance.

In the experiments described in this thesis, statistical inference has been applied to images using Gaussian random field theory (GRF) or cluster detection a specific **z** values.

A cluster based approach is considered to be superior to voxel based approaches in that it is more physiologically sound as activation patterns within the brain extend over a number of voxels and therefore should not be tested as independent. Cluster based approaches have also been shown to be more sensitive to change than voxel based approaches (Smith 2001).

In order to determine whether one PE or explanatory variable is more relevant to the data, or, in other words, better explains the images produced one PE is subtracted from the other and then divided by the standard error of the subtraction.

4.1.5 Conversion of raw data

Data recorded from the scanner were processed using MRIConvert v2.0 (Smith 2007) a freeware software package that has been extensively tested, to convert the DICOM images obtained from the scanner to the Nifti format recognised by FSL.

Although laterality should be preserved, issues have arisen within the imaging community regarding preservation of laterality during conversion (FSL discussion list).

The conversion pipeline used for the data described here was therefore initially checked on a data set gathered from a short scanning paradigm involving a unilateral motor task known to produce robust contralateral activation patterns. A MR visible oil capsule was placed on the right side of the head and the resulting converted images checked to ensure reported laterality within FSL matched that which was seen of the oil capsule on visual inspection of the images.

4.1.6 Group analysis

The analysis techniques discussed thus far are initially applied on an individual subject basis. Multi subject or group analysis is then undertaken. Low-resolution fMRI data from individual subjects is first registered to their own high resolution structural scan using 7 degrees – of - freedom (dof) linear fit then registered to the MNI 152 standard brain template using a 12 dof linear fit.
Registration into standard space ensures that first (individual) level statistic maps are all in alignment.

There are two common approaches towards group analysis; (i) fixed effect (FE) and (ii) mixed effects (ME)

Fixed effects analysis assumes that the experimental effect on the BOLD signal is constant or 'fixed' across all subjects except for the influence of random noise i.e. it ignores cross subject or cross session variance. This makes the FE approach sensitive to extreme results from one or two individual e.g. in a group of seven subjects, one or two with very strong activation will skew the results as the data is averaged. The main disadvantage therefore of FE is that statistical inference is restricted to a select study population.

Mixed-effects (ME) analysis utilises both the FE variance (within-session across-time variances estimated in the first-level analyses) and random effects (RE) variance - an estimate of the inter-session or inter-subject variance

Group analyses performed on experiments reported in this thesis use FLAME (FMRIB's Local Analysis of Mixed Effects) (Smith et al., 2004) which implements a mixed effects (ME) model in a Bayesian framework. FLAME models and estimates the inter-subject random effects component of the mixed-effect variance.

4.1.7 Experiment one: Paradigm design

FMRI experiments will typically follow either a block design whereby a stimulus is presented repeatedly over a prolonged but fixed period of time followed by a period of rest. Blocks are then repeated a number of times. Event related designs utilise discrete, short duration events or stimuli. The order and timing of which may be randomised and the interstimulus interval varied.

The experiment which follows utilised an atypical block design; atypical in that time periods of blocks were longer than that frequently reported elsewhere in the literature.
There exists a relationship between the intensity of pain induced by hypertonic saline and the time frame in which the pain subsequently abates. Although not strictly linear, the correlation is positive; that is in order to induce a pain of sufficient intensity one must accept the accompanying temporal aspects.

It was not possible to induce muscle pain via hypertonic saline injection that lasted for a shorted period of time than that stated whilst ensuring pain of sufficient intensity resulted.

The aim of this experiment was to investigate differences between brain activation patterns during muscle and cutaneous pain therefore the fMRI paradigm design implemented had to be selected based on the restrictions imposed by the experimental model of muscle pain.

4.2 Psychophysical data

As previously discussed a number of factors can affect the perception of pain either enhancing or reducing it. The scanning environment itself may contribute to this as demonstrated by Boyle et al., (2006) where reported VAS scores of pain

and unpleasantness were reduced by the simple addition of the noise of the scanner.

Additionally although confident that the hypertonic saline would reliably induce pain based on the psychophysical testing detailed in chapter three there remained some variation between injections therefore it was deemed imperative that online ratings of pain were gathered.

The choice to undertake both intensity and unpleasantness ratings may be questioned, given that the results from chapter three indicate a strong correlation between these two measures for both muscle and cutaneous pain. However the decision to gather both unpleasantness and intensity data online was again based upon the theoretical possibility that the scanning environment may affect the perception of either or both of these qualities.

A key finding from the psychophysical data reported in chapter three was that cutaneous and muscle pain could clearly be differentiated through the selection of sensory descriptors on the Short Form (SF) McGill pain questionnaire; therefore to ensure that the scanning environment itself did not corrupt that aspect of the pain sensation online SF McGill data was also gathered.

All the psychophysical data were therefore collected simultaneously whilst subjects were being scanned in order to (i) ensure subjects were experiencing pain and (ii) enable correlation in pain and unpleasantness scores with brain activation patterns

The in-house designed VAS device previously described in chapter three was therefore utilised throughout each scan. Sensitivity of the pressure pad was adjusted for each subject to ensure that a maximum score could be reached with minimum motor effort whilst minimum scores could also be reported. The device was also utilised to both determine unpleasantness ratings and to score the Short Form McGill Pain Questionnaire.

4.3 Methodology: Experiment one

An atypical extended block fMRI paradigm was utilised. Pain was induced in six blocked periods, each lasting approximately six minutes with rest periods within and between the blocks. During each block subjects received instructions to either focus on the pain, rate intensity or unpleasantness of the pain or rate words from the SF McGill Pain Questionnaire.

4.3.1 Subjects

Eighteen healthy volunteers (7 male, 11 female) mean age 36 (range 19 -60) participated in the study. All subjects were right-handed (by self-report). Subjects had near - normal or corrected-to-normal (with contact lenses) visual acuity. This was important to ensure that subjects were able to read instructions displayed to them during the scan.

Subjects were recruited via adverts to staff of The Walton Centre for Neurology and Neurosurgery and via The University of Liverpool intranet targeting both staff and students.

The South Sefton local research ethics committee and The Walton Centre for Neurology and Neurosurgery and Aintree Hospital (NHS) Trust Research Governance committees approved the study protocol.
Written informed consent was obtained from all subjects prior to enrolment in the study after provision of both written and verbal explanations of the protocol.

A semi-structured interview was undertaken in each individual; this consisted of past medical history and current symptoms. Subjects were excluded if there was (i) history of a cervical and /or upper limb musculoskeletal condition that had at any stage required investigations or treatment, (ii) history of skin disease (e.g. psoriasis, eczema), (iii) history of injury of upper arm or neck, (iv) ongoing current musculoskeletal symptoms (e.g., pain, weakness, stiffness), (v) any

persistent pain (including chronic daily headaches; however occasional migraine attack were allowed), (v) any systemic disease (except mild hypertension well controlled with medication), (vi) any chronic allergic conditions (e.g., urticaria; however, allergic rhinitis was accepted), (vi) history of a psychiatric or neurological condition, (vii) history or suspicion of current ongoing abuse of alcohol or drugs, (viii) current use of medications with analgesic properties even if used for other purposes, e.g. tricyclic antidepressants or anti-inflammatories), (ix) any ongoing non-drug therapy with analgesic properties (e.g., acupuncture for smoking cessation, aromatherapy for allergy).

Subjects were also screened for safety reasons by a radiographer to ensure there were no contraindications to undergoing MR scanning.

4.3.2 Pain induction and psychophysics evaluation

A total of four disposable plastic catheters (Vygon, 26 g, 25mm) were inserted in to the posterior aspect of the non-dominant forearm at two separate sites; extensor digitorum muscle belly and 1cm distal to this into intradermal tissue. These were inserted utilising an aseptic technique in a treatment room adjacent to the scan room.

Here the subjects were also familiarised with the online electronic VAS and the SF McGill pain questionnaire and informed that ratings of both intensity and unpleasantness would be requested. The difference between intensity and unpleasantness was explained by again using the verbal description of Price et al (1987) detailed previously in chapter three.

The fixed VAS end points representing 'no pain' and 'worst possible pain' during intensity rating, 'not at all unpleasant' and 'the most unpleasant sensation imaginable' during pain unpleasantness rating and 'none' and 'severe' during the SF McGill.

Subjects were asked to recount back to the investigator their own interpretation of the difference between intensity and unpleasantness to ensure the concept was understood. They were asked to utilise the full length of the scale as it reflected the sensation they were experiencing.

4.3.3 Scanning procedures

Once in the scan room the subjects were again familiarised with the electronic VAS and sensitivity was adjusted to ensure each subject could utilise the full extent of the scale with minimal motor effort.

In order to control for attention effects, instructions were displayed throughout the whole scanning duration on a screen visible to the subject via a mirror placed above the head coil. Instructions were to either 'focus on your pain', 'rate pain intensity', 'rate pain unpleasantness', 'rate pain words' – followed by each word displayed for 9s, or 'rest'.

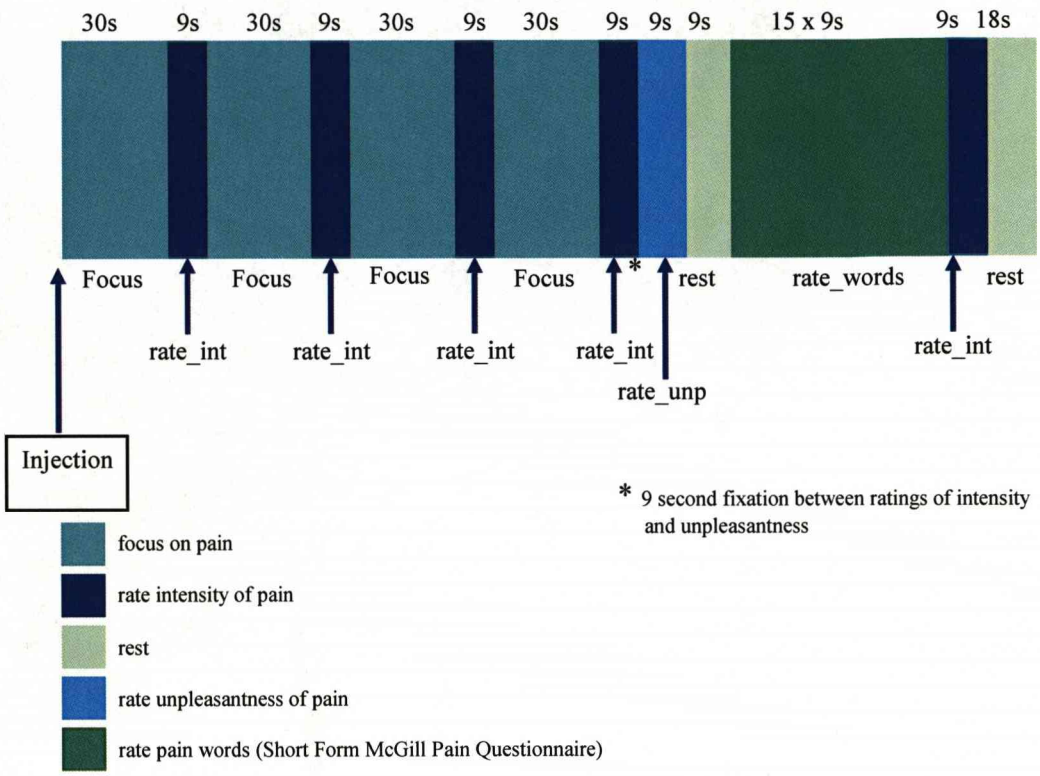
'Focus on your pain' was displayed in 4 x 30 seconds blocks from moment of injection with 9 second breaks for intensity ratings, followed by an unpleasantness rating.

A rest period of 18 seconds both preceded and followed the pain word ratings; immediately prior to the second rest period a final intensity rating was also obtained to ensure pain levels had reduced to a negligible level prior to proceeding to the next injection. Total scan time was approximately 36 minutes.
Figure 4.1 below outlines the timings of one 'block' of the paradigm.
Each subject received a total of six injections of 6% hypertonic saline, via extension leads connected to the cannulae, at intervals of six minutes in one scanning run.

Injections alternated between muscle and cutaneous sites; subjects were blinded to both the order and to which site was being injected. As the injections were given via extension tubing, the subjects also had no awareness of when a new injection was given or about to be given. Volume of injection required to produce a

moderate short lasting pain was previously determined in the laboratory setting; these were 0.35ml for muscle and 0.2ml for cutaneous.

Figure 4.1 Scanning paradigm timings of one block



4.3.4 MRI procedure and image acquisition

MR images were acquired using a 3T Trio MR Scanner (Siemens, Erlangen, Germany) using a BOLD (blood oxygen level dependent) sensitive T_2 * -weighted multislice gradient echo-planar imaging (EPI) sequence (echo time, 35 ms; repetition time, 3 s; flip angle, 80°; field of view, 224mm; slice thickness, 3.5 mm). Thirty contiguous axial slices were prescribed covering the whole brain. A total of 730 EPI volumes were collected.

Anatomical scans for co-registration of functional data and visualisation purposes were also obtained; a high resolution T_1-weighted 3D inversion recovery prepared

gradient echo sequence was acquired (TE = 5.4ms, TR = 12.3 ms, TI = 450ms, 1mm slice thickness, FOV = 20 cm, 256 x 192 matrix), with 192 axial slices.

4.3.5 Analysis of Imaging Data

4.3.5.1 Initial procedures

After converting scanner data from Dicom utilising MRIConvert v 2.0 (Smith 2002) the resulting FSL compatible Nifti 4d images were loaded into FSLview and observed in movie mode to look for any obvious artefacts or excess motion, the latter particularly in view of the length of the scan.

Whilst some head motion was observed in a number of subjects this was not excessive except in one subject in whom it was noted in particular excess head motion throughout most of the scanning period. Although initial analysis on an individual basis on this subject was undertaken the McFLIRT motion correction analysis confirmed excessive head motion throughout the scan therefore this subject was excluded from further analysis.

4.3.5.2 Brain extraction

Brain extraction was carried out on the subjects' structural images using FMRIBs brain extraction tool (BET2) (Smith 2002); deleting non-brain tissue from the image using a surface model approach. The structural images are implemented in the later stages of FEAT for registration purposes of functional data and then for co-registration with the MNI standard template brain. Successful registration is enhanced by improved BET outputs (Smith 2002).

4.3.5.3 Initial modelling of data

Although BET has been shown to be a reliable and valid too for brain segmentation (Smith 2002), a visual inspection was carried out by loading the BET output images into fslview. Two subjects were deemed to have less than ideal outputs with excess skull tissue remaining and were therefore re-processed implementing a vertical gradient of the fractional intensity threshold of 0.6 (from

the default of 0.5) which resulted in structural brain images with minimal skull tissue remaining.

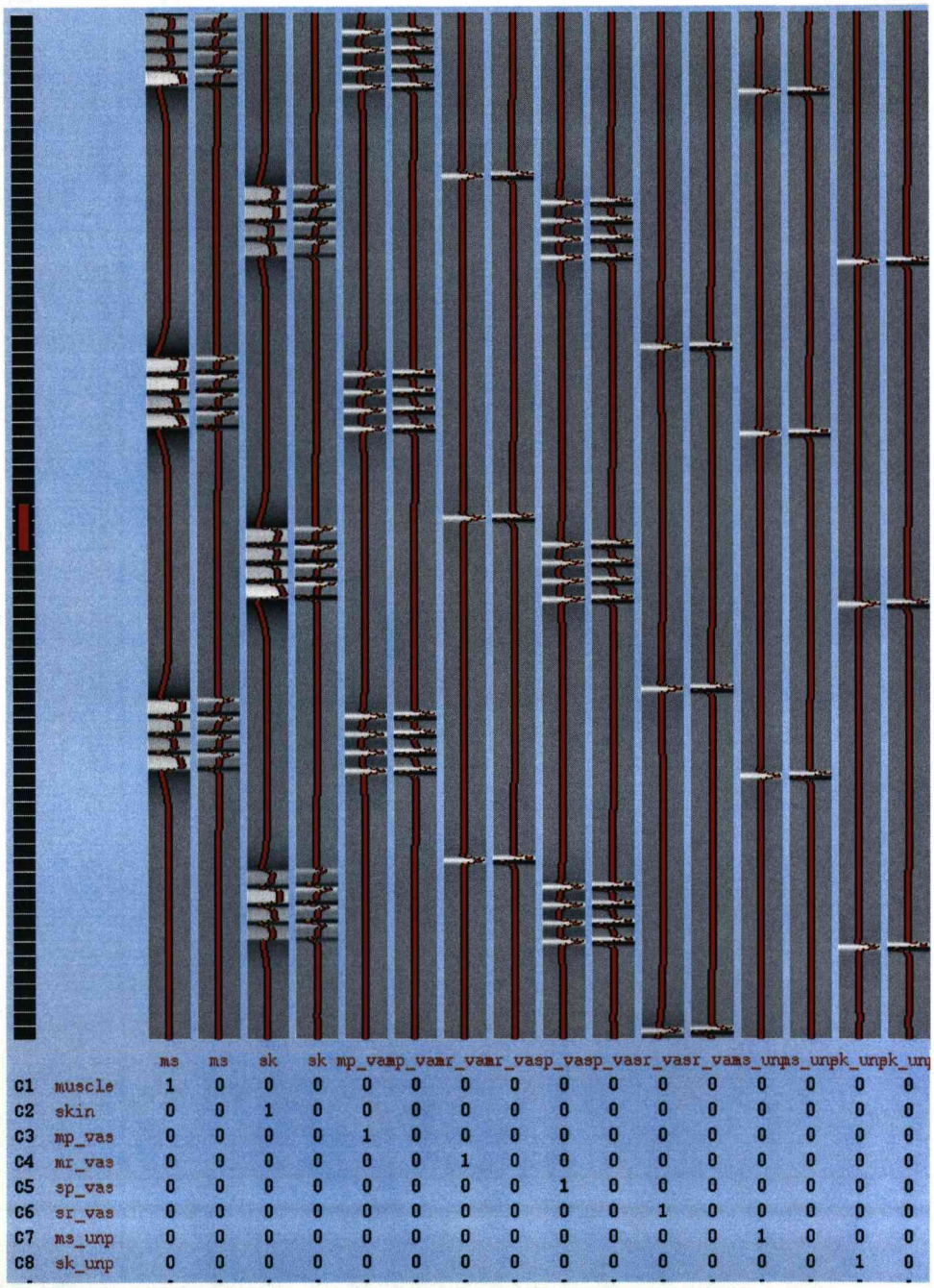
Pre-processing steps were applied to each functional dataset: spatial smoothing (Gaussian kernel, full width at half-maximum: 5 mm), motion correction and non-linear high-pass temporal filtering (sigma: 15 s).

A general linear model (GLM) was applied on a voxel by voxel basis to these data (Worsley and Friston, 1995) using FILM (FMRIB's improved linear model) (Woolrich et al., 2001) to model blood-oxygen-level-dependent (BOLD) signal intensity changes in response to painful stimuli. This first level analysis was undertaken on an individual basis of all subjects, including all data points, without reference to any psychophysical data.

The subject level statistical images were registered into MNI (Montreal Neurological Institute) standard space using FLIRT (Jenkinson and Smith, 2001). Separate explanatory variables (EVs) for inclusion were modelled for five conditions; muscle pain, cutaneous pain, attention to pain and attention to unpleasantness; the later two each having two separate EVs, one for muscle and one for cutaneous pain. Each regressor was constructed by convolving a boxcar function with a gamma haemodynamic response function. Voxel-wise parameter estimates (PEs) were derived for each regressor and for each subject a statistical image was calculated for each EV. The FSL model is shown in figure 4.2 below.

The same analyses were carried out using negative contrasts which tests for decreases in the BOLD signal. To explore further potential differences between intensity and unpleasantness, additional regressors were added to the model consisting of the de-meaned intensity and unpleasantness scores.

Figure 4.2 FSL design matrix used to model the individual first level analysis.



C1 muscle; C2 cutaneous; C3 muscle intensity during pain; C4 – muscle intensity during no pain; C5 cutaneous intensity during pain; C6 – cutaneous intensity during no pain; C7 muscle unpleasantness; C8 cutaneous unpleasantness;
For second level analysis, muscle and cutaneous events where VAS was < 3 were included in the model as EV's of no interest.

4.3.5.4 Second analysis

One of the advantages of obtaining online psychophysical data was the ability to obtain real time information regarding actual pain scores instead of relying upon off line pre-testing or post scan questionnaires.

On scrutinising the pain intensity data it was apparent that not all subjects experienced at least 'moderate' pain scores during all injections.

As a main objective of this study was to compare cutaneous and muscle pain when induced by the same method and of similar intensities only those events where intensity and unpleasantness of pain was rated as ≥ 3 during both muscle and cutaneous pain were included in the analysis; for this reason two subjects were excluded. SF McGill data was also scrutinised to ensure that word selection reflected the results obtained during non-scanning conditions for muscle and cutaneous pain. One further subject was excluded from the final analysis due to excessive head motion throughout the scanning period. A total of 15 subjects therefore contributed to the group analysis.

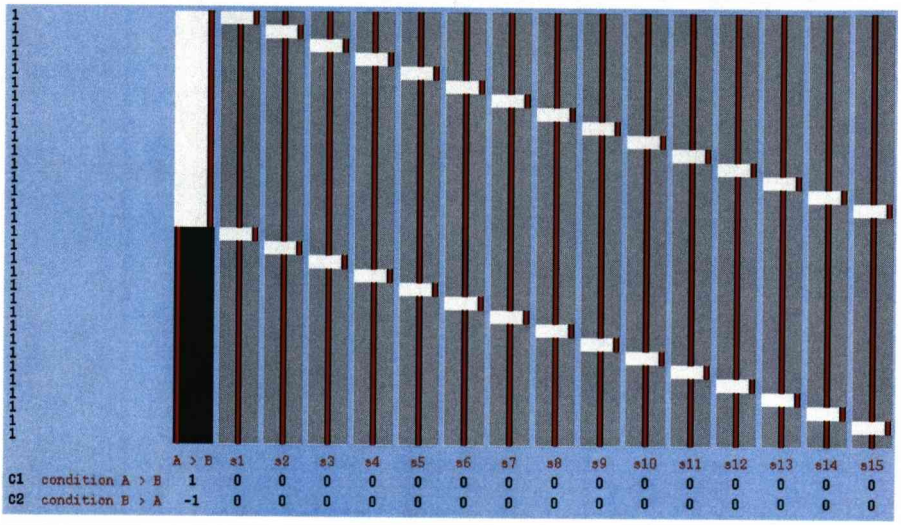
The subject level statistical images were registered into MNI (Montreal Neurological Institute) standard space using FLIRT (Jenkinson and Smith, 2001). For each subject, one statistical image was calculated each for muscle and for cutaneous pain conditions which met the inclusion criteria.

Group analyses were performed using FLAME (FMRIB's Local Analysis of Mixed Effects) (Smith et al., 2004) which implements a mixed effects model in a Bayesian framework. FLAME models and estimates the inter-subject random effects component of the mixed-effect variance. Group statistical maps were thresholded at $Z = 2.3$ with significant clusters defined according to spatial extent at $P < 0.01$ (corrected for multiple spatial comparisons according to Gaussian random field theory (Worsley et al., 1992).

Group statistical maps were calculated using one regressor constant across subjects for each condition to determine a group activation map for each

condition. Two-sample paired T-Tests were then implemented within FLAME to determine muscle – cutaneous differences; the FSL design matrix for this is shown below in figure 4.3.

Figure 4.3 FSL design matrix used to model the group contrasts muscle > cutaneous and cutaneous > muscle.



Condition A = muscle; condition B = cutaneous.

EVs s1-s15 model each subject's mean effect and are added as EV of no interest to the model to ensure that the individual mean effects do not interfere with the estimation of the A-B paired differences (Smith 2001)

4.3.5.5 Region of interest analysis

In order to test further the hypothesis that muscle pain, having a greater affective component, would demonstrate activation in more rostral areas of the anterior insula a region of interest analysis (ROI) was carried out to determine co-ordinates of peak activation on an individual basis.

Featquery is a tool within FSL that allows further interrogation of FEAT results in a user defined mask or set of co-ordinates to determine mean stats values (Smith 2001). Two ROIs were drawn onto the MNI template brain; the anterior insula (insula anterior to the central sulcus) was subdivided into a caudal and a rostral part. The middle and posterior short gyrus was defined as caudal anterior insula (cAI) and the anterior short gyrus as rostral anterior insula (rAI) following the

subdivision previously published by Schweinhardt et al (2006). The anterior limit of the short anterior gyrus is the anterior limiting sulcus; the posterior short gyrus is limited posteriorly by the central sulcus of the insula. Anterior and middle short gyri are separated by the short insular sulcus.
Featquery was then run on each subject's first level analysis results; only those events where pain was >3 were included.

4.4 Results

4.4.1 Psychophysical data

The results presented include only those taken during injections which were included in the fMRI analysis as discussed above.

Peak VAS score during muscle pain ranged from 3 - 9.8 (mean 4.4, sd 2.5) and from 3 - 9 (mean 4.9, sd 2.1) during cutaneous pain. There was no significant difference between the peak VAS scores of the two pain conditions ($p>0.05$). The course of pain onset and offset is shown in fig. 4.1 below.

Unpleasantness scores during muscle pain ranged from 3 - 10 (mean 3.9, sd 2.7) and from 3 – 9.7 (mean 4.3, sd 2.5) during muscle and cutaneous pain respectively. No difference in unpleasantness rating ($p>0.05$) was found between ID and MS across all six injections

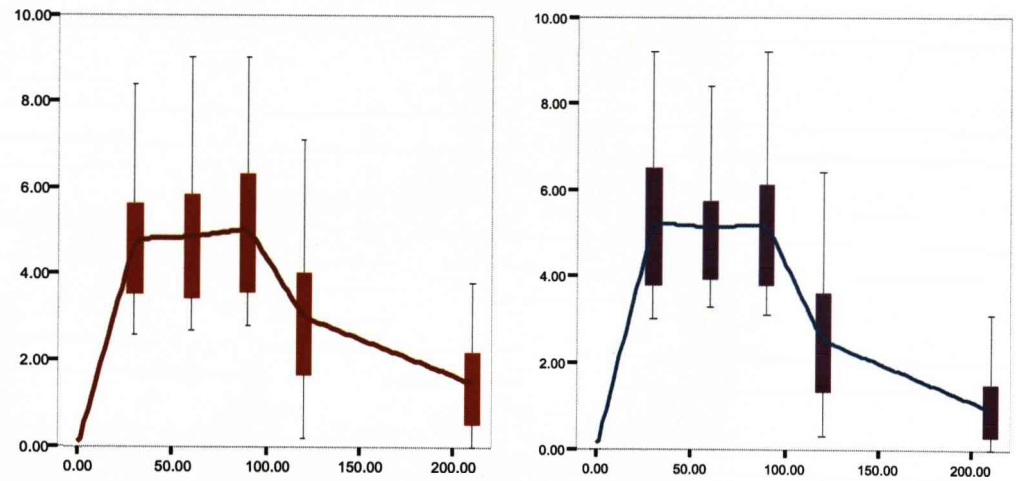
Unpleasantness was found to correlate with intensity (Pearsons $r = .83$, $r = .88$, $p < 0.01$) for muscle and cutaneous pain respectively.

The words hot burning / stabbing / sharp / shooting were selected more frequently and scored higher in the ID condition ($p<0.05$). During MS condition throbbing / gnawing / aching / heavy and cramping were selected more frequently and scored higher ($p<0.05$).

Table 4.1 Psychophysical results

	N		
	Statistic	Mean	Std. Deviation
Peak intensity			
ID	33	4.9	2.1
MS	33	4.4	2.5
Unpleasantness			
ID	33	4.3	2.5
MS	33	3.9	2.7

Figure 4.4 Mean VAS scores of muscle pain (A) and cutaneous pain (B)



A. Time (s) B. Time (s)

4.4.2 fMRI results

4.4.2.1 Similarities

Both cutaneous and muscle pain was associated with an increase in BOLD signal in bilateral primary sensory and motor cortices (SI and MI) and anterior and posterior insula cortices. Bilateral anterior cingulate signal increase was also present with both conditions demonstrating separate peaks of activation in the pMCC and aMCC.

4.4.2.2 Differences: laterality

In a number of brain regions bilateral signal increase was observed during muscle whilst during cutaneous pain this was only contralateral (secondary sensory cortex (SII), the cingulate motor area (CMA), orbito-frontal cortex and the putamen) or ipsilateral (DLPFC, VLPFC, PCC).

4.4.2.3 Differences: muscle versus cutaneous pain

Areas where signal increase occurred only in muscle pain were bilateral thalamus and MPFC, and contralateral perigenual ACC, caudate nucleus and cerebellum. Peak activations are reported in Table 4.2 and areas of activation figures 4.5 and 4.6.

The contrast of muscle>cutaneous showed ipsilateral pACC and contralateral pACC and caudate nucleus activation. Cerebellum and MPFC did not survive thresholding at $z > 2.3$ however both showed sub threshold differences at $z = 2.03$ and $z = 1.9$ respectively. These results are also shown in table 4.2 and figure 4.7.

No activation survived the cutaneous > muscle contrast.

Table 4.2 Areas of activation during muscle pain, cutaneous pain and the muscle – cutaneous contrast.

Brain region	Muscle			Cutaneous			Muscle – cutaneous contrast		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x, y, z	Z score
ACC – aMCC	I	-0, 26, 28	3.5	I	-6, 18, 36	2.88	I	-2, 28, 28	2.3
	C	2, 24, 24	3.4	C	4, 18, 26	2.69	C	2, 40, 16	2.42
ACC – pMCC	I	-12, 8, 40	3.2	I	-4, 4, 44	2.41	I		
	C	12, 6 34	2.83	C	2, 4, 44	3.05	C		
ACC – CMA	I	-2, 4, 36	3.5	I			I	-2, 8, 40	2.3
	C	2, 4, 38	3.3	C	4, -10, 40	2.64	C		
ACC perigenual	I			I			I	-8, 40, 6	
	C	6, 34, -2	2.7	C			C		
PCC	I	-2, -19, 36	2.7	I	-2, -16, 42	2.35	I		
	C	2, -18, 38	2.69	C	4, -16, 44	2.71	C		
Anterior insula	I	-36, 18, -4	3.56	I	-44, 4, -8	3.32	I	-28, 18, 6	3.2
	C	36, 20, -6	3.56	C	32, 14, 0	2.41	C		
Posterior insula	I	-34, -8, 4	3.5	I	-40, -6, -4	3.56	I		
	C	36,-10,2	4.94	C	40, -4, 12	4.4	C		
Thalamus	I	-8, -16, 6		I			I		
	C	8, -8, 6	2.42	C			C	8, -22, 12	2.4
MI	I	-4,-28,58	3.22	I	-2, -26, 54	2.69	I		
	C	10,-26,64	4.54	C	10, -26, 64	4.64	C		
SI	I	-8,-42,54	2.67	I	-54, -20,	4.25	I		
	C	10, -36, 60	3.26	C	54, -10, 32	4.02	C		
SII	I	-54, -4, 12	3.8	I			I	-56, -4, 10	3.3
	C	50, -20, 16	3.2	C	54, -10, 10	4.02	C		
Cerebellum	I			I			I		
	C	24, -76, -26	3.25	C			C	22,-76, -24	*2.03
Putamen	I	-28, 8, 0	2.77	I			I	-24, 12, 6	2.5
	C	26, 8, 0	3.92	C	26, 14, 4	4.64	C		
Caudate nucleus	I			I			I		
	C	16, 16, 2	3.25	C			C	14, 20, 0	3.5
DLPFC	I	-36, 36, 18	3.78	I	-36, 32, 22	3.49	I	-32, 38, 40	3.6
	C	34, 36, 16	3.96	C			C	36, 38, 18	2.35
VLPFC	I	-40, 42, -4	3.36	I	-46, 40, 4	3.48	I	-36, 42, 16	2.36
	C	40, 42, -4	3.6	C			C	42, 40, 2	3.8
MPFC	I	-10, 38, -8	2.55	I			I	-12, 46, -2	1.9*
	C	10, 36, -6	2.95	C			C		
Orbitofrontal	I	-38, 38, -8	3.28	I			I		
	C	36, 34, -6	3.4	C	34, 26, -4		C		

MPFC and cerebellum activation in contrast muscle > cutaneous just below set threshold of Z > 2.3

Figure 4.5 Areas of activation observed during muscle pain

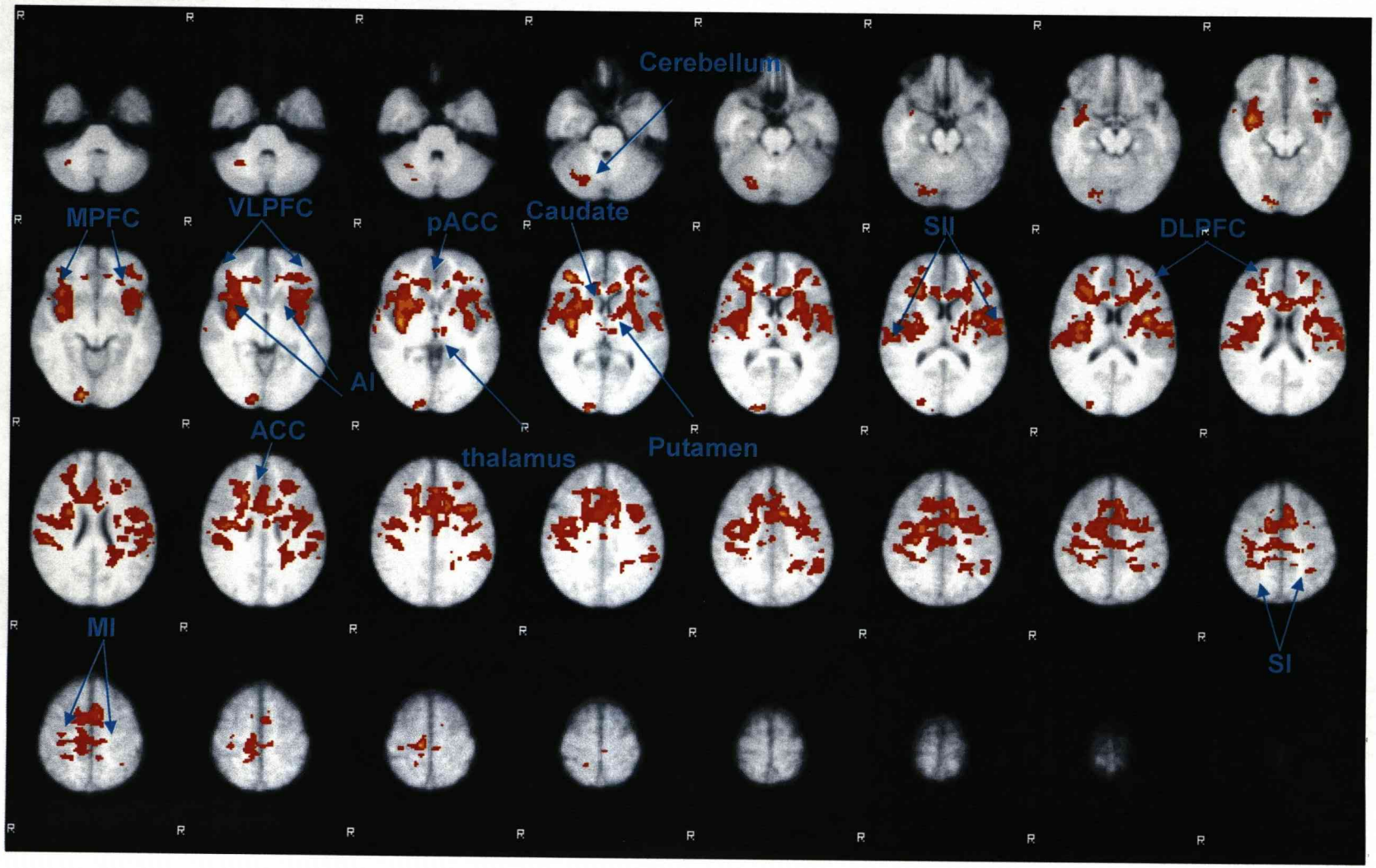


Figure 4.6 Areas of activation observed during cutaneous pain

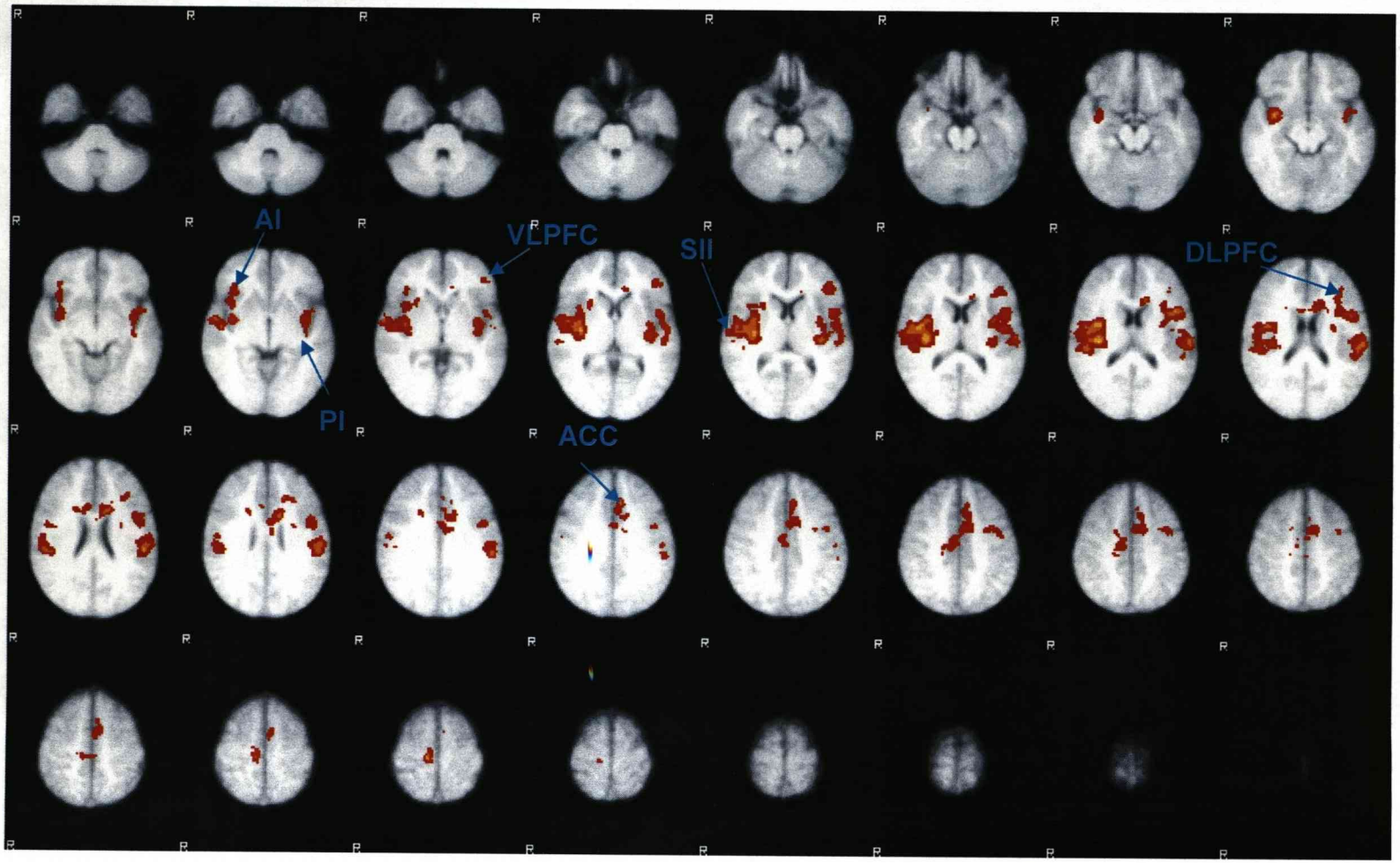
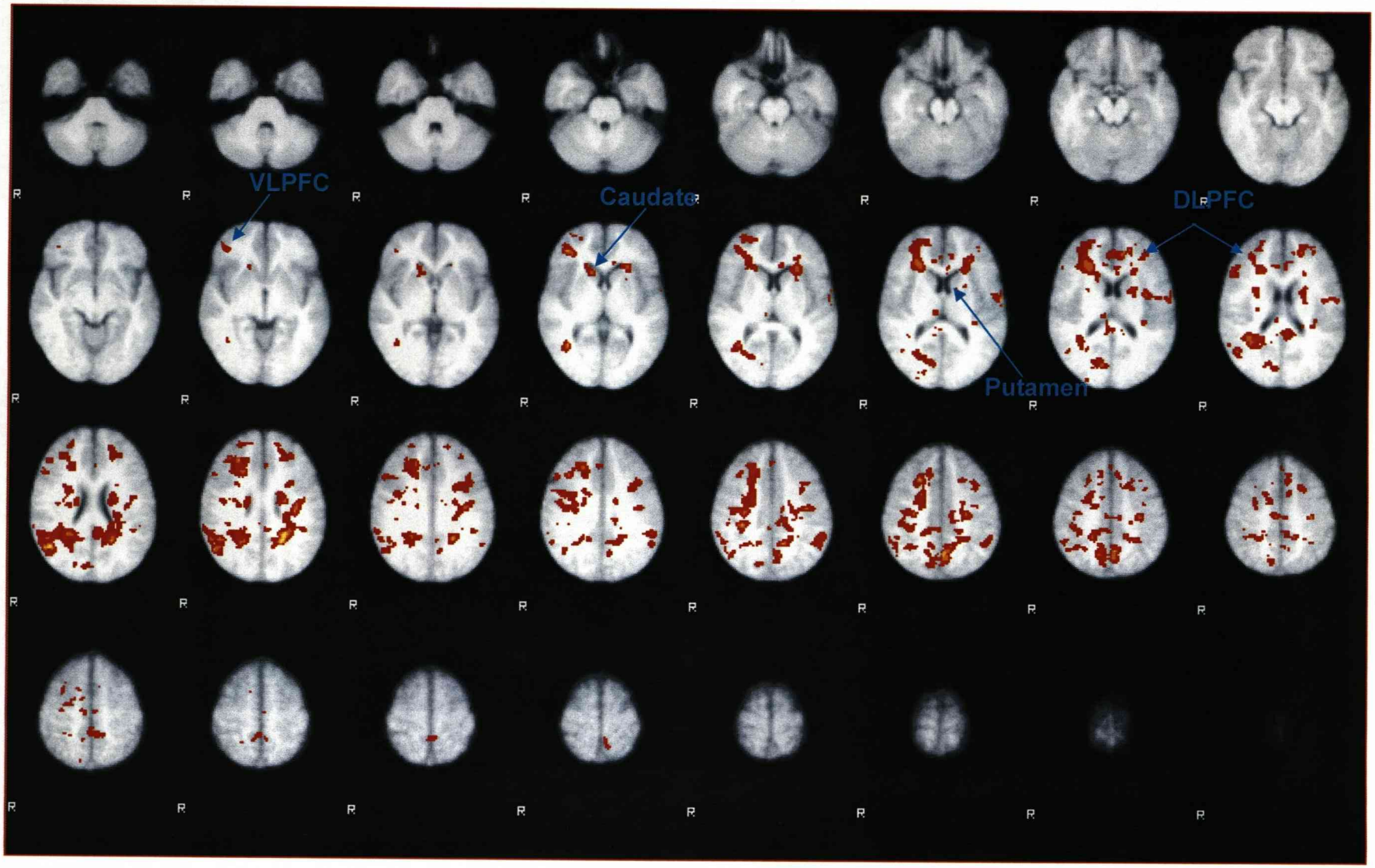


Figure 4.7 Activation map of the contrast muscle pain > cutaneous pain



4.4.2.4 Attention to intensity

During attention to / rating of intensity increases in BOLD signal were seen bilaterally in ACC and SMA and ipsilateral in MI and SI in both conditions. Bilateral putamen activation was also observed during attention to intensity of muscle but not cutaneous pain. This putamen activation however did not survive a muscle - cutaneous contrast. Bilateral ACC and SMA and ipsilateral MI and SI activation showed a positive linear variation with intensity ratings; no differences were seen between the two conditions in terms of intensity co-variance.
Co-ordinates of peak activations are reported in Table 4.3; those areas where activation co-varied with intensity score are shown in table 4.5.

Table 4.3 Activation during attention to intensity rating

Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x, y, z	Z score
aMCC	I	-4, 18, 34	3.25	I	-2, 28, 30	2.72
	C	6, 16, 34	3.13	C	8, 2, 38	3.1
Anterior insula	I	-32, 22, -4	3.71	I		
	C	30, 20, 6	3.88	C		
MI	I	-30, -18, 58	4.85	I	-30,-18, 58	4.12
	C	30, -6, 50	2.87	C		
SMA	I	-6, -2, 58	3.98	I	-4, -2, 56	3.48
	C	6, -2, 54	3.31	C	4, 4, 52	2.83
SI	I	-44, -30, 48	4.28	I	-46, -28, 48	
	C			C		
Putamen	I	-22, 6, -2	3.15	I		
	C	24, 6, 2	3.51	C		

*** No activation survived the muscle > cutaneous or cutaneous > muscle contrast**

4.4.2.5 Attention to unpleasantness

During attention to unpleasantness an increased number of brain regions were activated including bilateral putamen, pallidum and prefrontal cortex. Those areas showing a positive linear relationship with unpleasantness ratings included bilateral ACC and MI, ipsilateral anterior insula, OFC and cerebellum and contralateral DLPFC during both conditions. Additional areas showing a positive linear relationship during muscle pain only included ipsilateral caudate nucleus and putamen and bilateral VLPFC.

A decrease in BOLD signal that co-varied linearly with unpleasantness scores during attention to unpleasantness in the muscle but not cutaneous condition was also seen in bilateral amygdala and contralateral cerebellum and posterior insula.

Co-ordinates of peak activations are reported in Table 4.4; those areas where activation co-varied with unpleasantness score are shown in table 4.6. See also figures 4.8, 4.9, 4.10)

Figure 4.8 Decrease in activation in the amygdala bilaterally during attention to unpleasantness during muscle pain which correlated with unpleasantness score

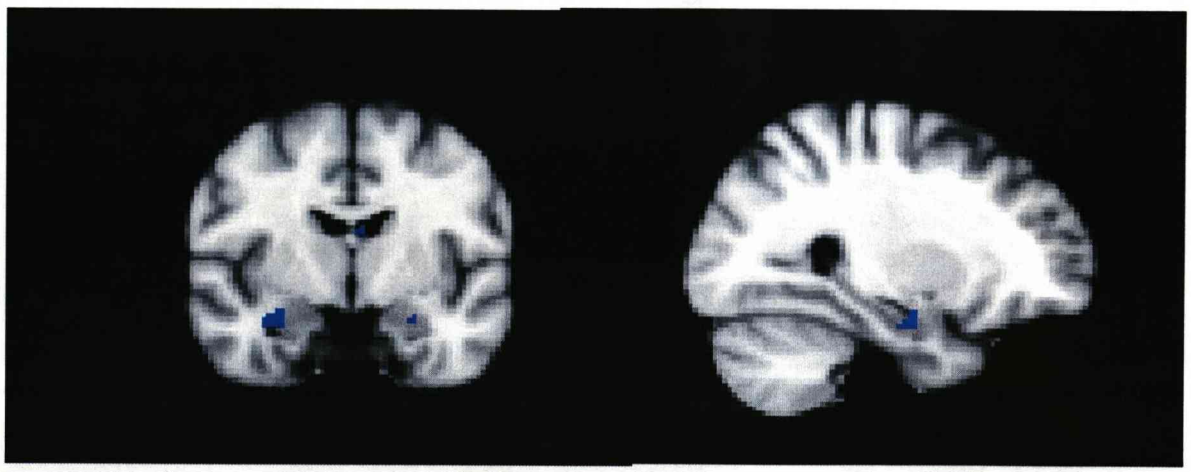


Table 4.4 Activation during attention to unpleasantness rating

Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x,y,z	Z score
ACC - aMCC	I	-4, 24, 32	3.13	I	-6, 20, 34	2.56
	C	2, 18, 36	3.9	C	2, 18, 36	3.57
ACC – pMCC	I	-4, 4, 40	3.33	I	-2, 0, 44	3.32
	C	2, 6, 44	3.6	C	2, 6, 42	3.51
PCC	I			I		
	C	2, -38, 30	2.56	C		
Anterior insula	I	-32, 22, -6	3.67	I	-32, 22, -2	3.56
	C	34, 22, -6	3.7	C	32, 22, -6	3.69
Thalamus	I	-10, -18, 8	3.38	I	-18, -2, 8	3.89
	C	10, -14, 2	3.01	C	12, 0, 10	3.47
MI	I	-42, -4, 42	5.02	I	-40, -2, 42	4.63
	C	42, 0, 46	3.9	C	38, -8, 54	3.94
SI	I	-44, -30, 50	3.84			
	C					
SII	I	-54, -22, 26	2.96			
	C					
Cerebellum	I	-30, -60, -26	2.82	I	-36, -64, -30	2.56
	C	34, -60, -36	3.05	C		
Putamen	I	-16, 8, -2	3.91	I	-26, 4, 12	3.13
	C	18, 10, -2	3.86	C	16, 10, 2	3.68
Pallidum	I	-18, 4, 0	3.58	I	-12, 4, 2	3.21
	C	16, 6, 0	3.42	C	22, -4, 0	2.78
DLPFC	I			I		
	C	34, 42, 18	3.39	C	44, 32, 12	3.35
VLPFC	I	-40, 42, 4	3.23	I		
	C					
Orbitofrontal	I	-48, 22, -2	2.88			
	C					

*** No activation survived the muscle > cutaneous or cutaneous > muscle contrast**

Figure 4.9 Activation which co-varied with unpleasantness scores during attention to unpleasantness of muscle pain

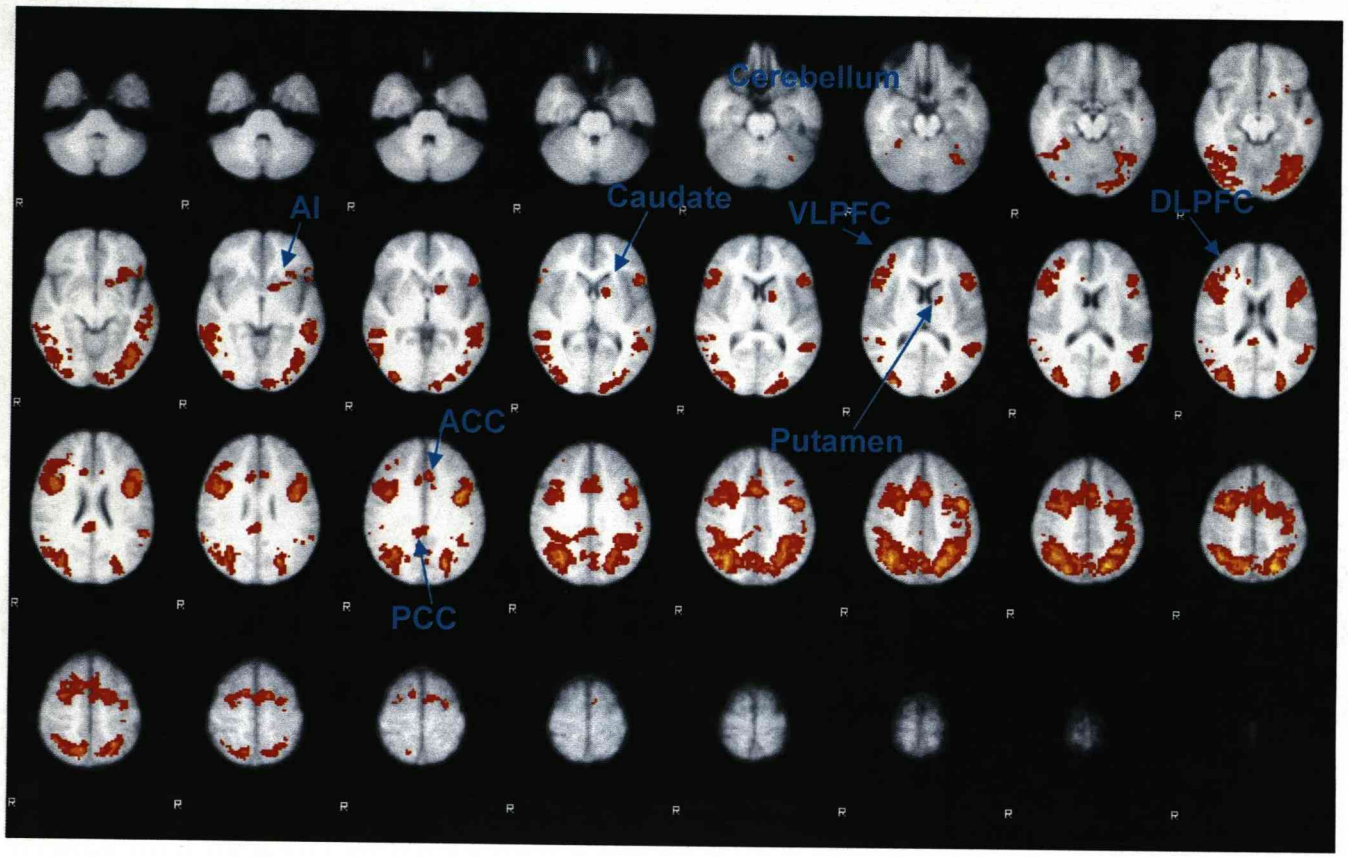


Figure 4.10 Activation which co-varied with unpleasantness scores during attention to unpleasantness of cutaneous pain

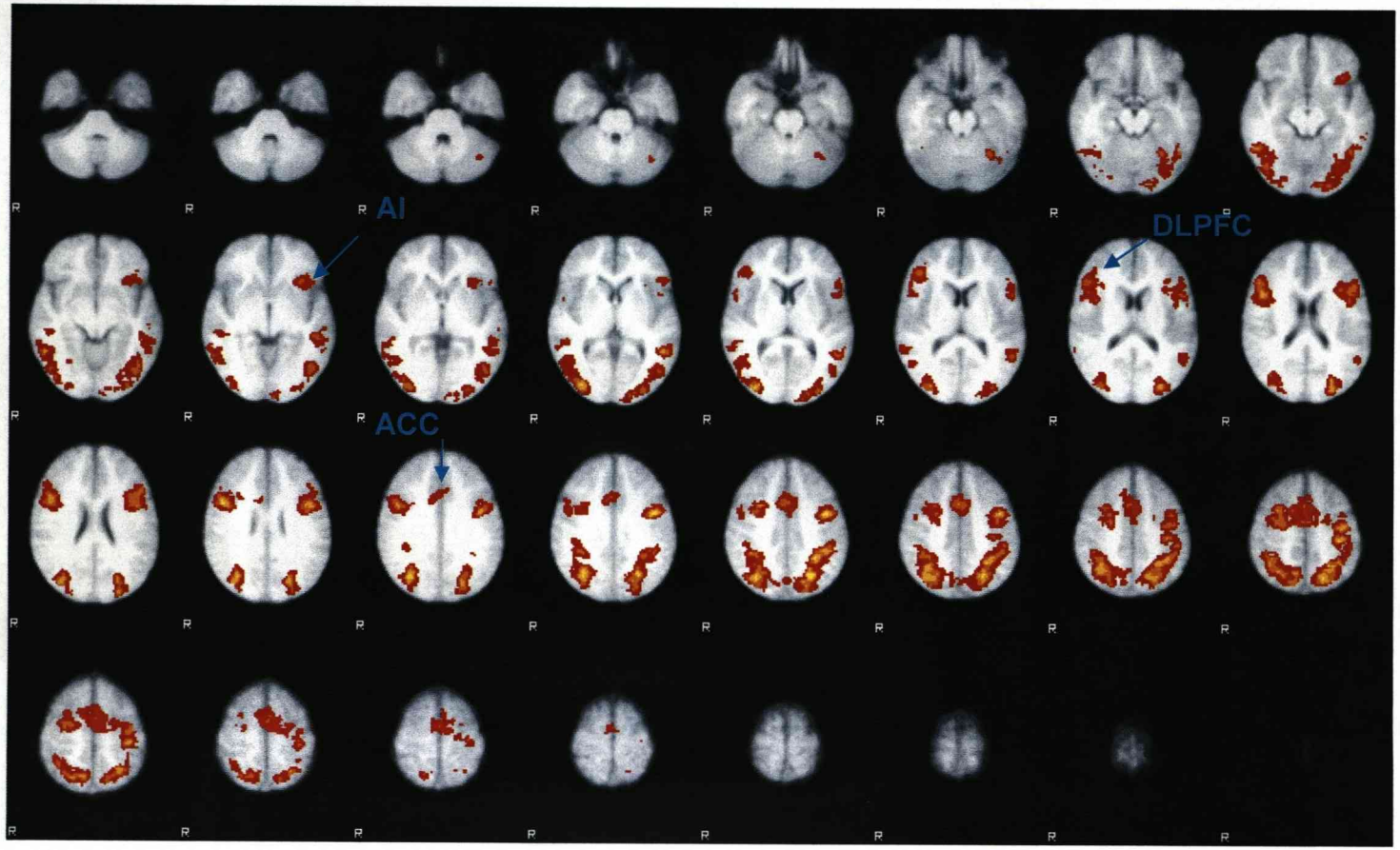


Table 4.5 Areas of brain activation during attention to intensity showing linear correlation with intensity vas

Brain area	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x, y, z	Z score
aMCC	I	-4, 20, 34	2.93	I	-6, 24, 34	3.14
	C			C		
pMCC	I	-2, 8, 38	3.5	I	-2, 6, 40	2.8
	C	4, 10, 38	3.51	C	2, 12, 40	3.1
SI	I	-34, -28, 54	4.22	I	-46, -36, 50	4.2
MI	I	-38, -18, 56	3.74	I	-32, -26, 50	4.29
SMA	I	-2, 0, 46	2.97	I	-2, -4, 52	2.82
	C	4, 2, 46	3.54	C	2, -10, 56	3.01

Table 4.6 Areas of brain activation during attention to unpleasantness showing linear correlation with unpleasantness vas

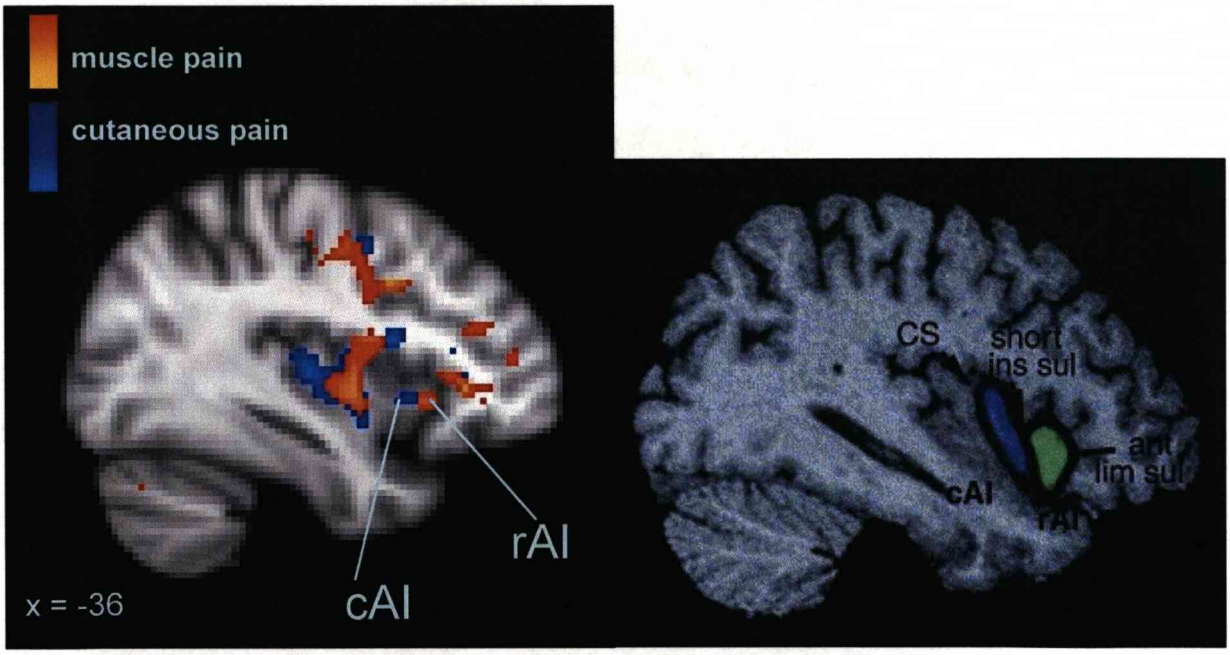
Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x,y,z	Z score
aMCC	I	-4, 24, 34	3.2	I	-2, 18, 36	2.8
	C	8, 26, 24	2.41	C	2, 16, 36	3.36
pMCC	I	-2, 8, 42	3.6			
	C	8, 10, 38	3.1			
PCC	I	-6, -38, 36	2.47			
	C	6, -38, 34	3.2			
Anterior insula	I	-30, 24, -8	3.25	I	-38, 16, -4	
MI	I	-38, 0, 42	4.33	I	-40, -10, 54	3.74
	C	44, -2, 42	3.41	C	38, -12, 54	3.71
SI	I	-36, -26, 54	2.53	I	-34, -28, 62	3.2
Cerebellum	I	-30, -60, -26	2.82	I	-38, -64, -30	2.98
Caudate nucleus	I	-12, 10, 2	3.02	I		
Putamen	I	-20, 10, -2	2.61	I		
DLPFC	I	-50, 18, 20	3.16	I		
	C	52, 24, 20	3.41	C	44, 34, 10	3.48
VLPFC	I	-52, 22, 6	3.36	I		
	C	50, 30, 8	3.18	C		
Orbitofrontal	I	-50, 26, -6	2.67	I	-44, 22, -8	2.52

4.4.3 Region of interest analysis

Activation was observed in the anterior insula during both muscle and cutaneous pain conditions Results from group analysis show that the co-ordinate of peak activation in the anterior insula during muscle pain was more rostral than it was during cutaneous pain (muscle; -36, 18, -4; 36, 20, -6., cutaneous: -44, 4, -8; 32, 14, 0). See Figure 4.11.

A paired t-test was carried out on the 'y' co-ordinates, comparing muscle and cutaneous pain and was found to be not significant ($p>0.05$).

Figure 4.11 Anterior insula activation during cutaneous and muscle pain



A: Anterior insula activation – group activation appears more rostral (rAI) during muscle than during cutaneous pain. (A)

B: Anatomical details of delineation of the rostral anterior insula (rAI, green) and caudal anterior insula (cAI, blue). rAI was defined to consist of the anterior short gyrus of the insula and cAI of the middle and posterior short gyrus. The short anterior gyrus is limited anteriorly by the anterior limiting sulcus; the posterior short gyrus is delimited posteriorly by the central sulcus of the insula. Anterior and middle short gyri are separated by the short insular sulcus. ant lim sul, anterior limiting sulcus; CS, central sulcus (of the insula); short ins sul, short insular sulcus. [Reproduced from Schweinhardt et al., 2006. Copyright (2006), with permission from Elsevier, see Appendix C]

4.5 Summary of fMRI results

4.5.1 Similarities

Both cutaneous and muscle pain were associated with an increase in BOLD signal in bilateral primary sensory and motor cortices (SI and MI) and anterior and posterior insula cortices. Bilateral anterior cingulate signal increase was also present with both conditions demonstrating separate peaks of activation in the pMCC and aMCC.

Activation in these areas of the brain has previously reported in a number of experimental pain studies (Derbyshire et al., 1997; Davis et al., 1998, Apkarian et al., 1999, Becerra et al., 1999, Gelnar et al., 1999).

This finding therefore provides support for the validity of the HS model as a method of pain induction within a scanning environment and also the paradigm employed.

4.5.2 Differences

In a number of brain regions bilateral signal increase was observed during muscle pain whilst during cutaneous pain this was only contralateral (secondary sensory cortex (SII), the cingulate motor area (CMA), orbito-frontal cortex and the putamen) or ipsilateral (DLPFC, VLPFC, PCC).

Previous reports in the literature suggest that an increasing number of brain areas in addition to increased bilateral activation occur as pain intensity increases (Peyron et al., 2000; Apkarian et al., 2005).

Similar levels of pain intensity were induced in both the muscle and cutaneous conditions in this study therefore greater intensity would not appear to explain the increase in bilateral activation in muscle compared to cutaneous pain observed here.

Areas where signal increase occurred only in muscle pain were bilateral thalamus and MPFC, and contralateral perigenual ACC, caudate nucleus and cerebellum. These brain regions have all been reported in previous brain imaging studies; the frequency of report however is rather less than it is for 'pain matrix' areas.

In the case of MPFC and perigenual cingulate, activation has been more frequently reported in clinical pain studies or experimental studies pain studies involving manipulation of attentional aspects; increased activity being associated with arousal related to emotional / motivational processing (Critchley et al., 2004).

4.5.3 Attention to intensity

During attention to / rating of intensity, increases in BOLD signal were seen bilaterally in ACC and SMA and ipsilateral in MI and SI in both conditions. Bilateral putamen activation was also observed during attention to intensity of muscle but not cutaneous pain.

Bilateral ACC and SMA and ipsilateral MI and SI activation showed a positive linear variation with intensity ratings; no differences were seen between the two conditions in terms of intensity co-variance.

Again these regions have all been implicated in pain intensity coding in previous studies (Peyron et al., 2000, Apkarian et al., 2005); providing further validation of the model chosen.

4.5.4 Attention to unpleasantness

During attention to unpleasantness an increased number of brain regions were activated including bilateral putamen, pallidum and prefrontal cortex. Those areas showing a positive linear relationship with unpleasantness ratings included bilateral ACC and MI, ipsilateral anterior insula, OFC and cerebellum and contralateral DLPFC during both conditions.

Additional areas showing a positive linear relationship during muscle pain only included ipsilateral caudate nucleus and putamen and bilateral VLPFC.

A decrease in BOLD signal that co-varied linearly with unpleasantness scores during attention to unpleasantness in the muscle but not cutaneous condition was also seen in bilateral amygdala and contralateral cerebellum and posterior insula.

4.5.5 Region of interest analysis

The anterior insula was a specific a priori area of interest in this study; it was hypothesised that HS induced muscle pain, having a greater affective component (Rainville et al., 1992) and showing behavioural characteristics of clinical musculoskeletal pain syndromes (Curatolo et al., 2000) would more closely resemble brain activation patterns observed in clinical pain condition.

However, despite the group activation maps indicating that the anterior insula activation was more rostral during muscle compared to cutaneous pain; subsequent ROI analysis revealed no significant difference between the two conditions in terms of co-ordinates of peak activation.

ROI analysis on an individual subject basis revealed distinct areas of activation during both conditions, in both rostral and caudal AI bilaterally. A possible explanation for this finding may be the tonic nature of stimulation employed in this study in comparison to the brief phasic pain typically employed in other experimental studies included in the review by Schweinhardt et al (2006).
Rostral anterior insula activation observed here, during both conditions may well be related to the inescapable / aversive nature of prolonged pain (Lumb et al., 2001).

It should also be noted that the ROIs drawn in this study are somewhat more conservative than those employed by Schweinhardt and colleagues (2006). Although based on the anatomical landmarks reported by those investigators; ROI's in this study were also guided by reference to MNI probalistic maps. It has been argued that this method is more accurate. On reproduction and visualisation

of Schweinhardt's (2006) previously published maps on the standard MNI brain in FSL view, the rostral map was observed to encroach onto the orbitofrontal cortex where activation was also seen. ROI analysis, as implemented by Featquery, searches for the peak of activation therefore the accuracy of the results are dependant on the accuracy of the map selected.
Therefore to avoid the confound of activation in the orbitofrontal cortex during ROI analysis where the primary question was regarding rostral anterior insula activation the more conservative route was employed

4.6 Key fMRI findings

Activation of areas of the 'pain matrix' was observed during both conditions providing support for the validity of the model employed.

FMRI results suggest patterns of brain activity associated with muscle and cutaneous pain induced by the same method, whilst sharing commonalities do also demonstrate differences which may warrant further investigation.

Changes in signal observed during muscle pain is consistent with the hypothesis that muscle pain engages more affective and emotional processing when compared to cutaneous pain.

Furthermore differences observed also provide support for the proposal that the experimental muscle pain model more closely reflects clinical pain than does the experimental cutaneous pain model.

Further interpretation, particularly in relation to the literature, and the implications of these results are discussed in chapter six.

Chapter five presents the results of two small fMRI experiments carried out with three patients with a diagnosis of unilateral epicondylitis (tennis elbow).
The first experiment implements the same methodology used in the previous chapter.

As small numbers do not allow for sensible statistical analysis the results are presented predominantly as a narrative with images for illustration purposes.

Despite the limited numbers, some interesting observations are made.

5.1 Background

The studies described thus far in this thesis have focussed on experimentally induced pain, addressing differences in the psychophysical properties and neural correlates between cutaneous and muscle pain. The relative strengths of experimental pain in terms of controllability, repeatability and reliability however also contribute to the weakness of experimental pain in relation to clinical pain.

As reported in Chapter 2, clinical pain conditions are inherently variable in nature due to both intrinsic and extrinsic factors. However increasingly investigators have realised that brain activation patterns observed during acute, physiological pain processing may not reflect that which happens in chronic pain conditions. Of late therefore researchers' have turned their attention to these patient populations although the overriding problem is the difficulty in assembling a homogenous patient cohort, particularly in respect to matching symptom profile, duration of disease, medication history and age distribution (May 2007).

This chapter describes two experiments carried out on three patients with a diagnosis of unilateral lateral epicondylitis (tennis elbow). This clinical condition was specifically chosen for a number of reasons including (i) it is a well defined clinical condition with known local tissue pathology involving deep tissue, (ii) it is typically unilateral, thereby allowing for the patient to serve as their own control (iii) it is frequently a self limiting condition that spontaneous resolves, or resolves with simple therapeutic interventions (Calfee et al., 2008).

5.2 Methods: Experiment one

5.2.1 Subjects

Three subjects (male, average age 40 (32- 47) took part in experiment one, which replicated the experiment described in chapter four in 18 healthy volunteers. Injections of hypertonic saline were administered as per the protocol outlined in Chapter four; the arm not affected by lateral epicondylitis was in this case injected.
In subjects A and B this was the left arm; in subject C this was the right arm.

5.2.2 Clinical examination

A semi-structured interview was undertaken in each individual; this consisted of past medical history and history of current symptoms; including any ongoing therapies.

Subjects were excluded if there was (i) history of a cervical and /or upper limb musculoskeletal condition that had at any stage required investigations or treatment other than their present condition, (ii) history of skin disease (e.g. psoriasis, eczema), (iii) history of injury of upper arm or neck, (iv) ongoing current musculoskeletal symptoms (e.g., pain, weakness, stiffness), (v) any persistent pain (including chronic daily headaches; however occasional migraine attack were allowed), (v) any systemic disease (except mild hypertension well controlled with medication), (vi) any chronic allergic conditions (e.g., urticaria; however, allergic rhinitis was accepted), (vi) history of a psychiatric or neurological condition, (vii) history or suspicion of current ongoing abuse of alcohol or drugs, (viii) (ix) any ongoing non-drug therapy with analgesic properties (e.g., acupuncture for smoking cessation, aromatherapy for allergy).
Subjects were also screened for safety reasons by a radiographer to ensure there were no contraindications to undergoing MR scanning.

This was followed by a physical examination to confirm diagnosis of lateral epicondylitis. Diagnosis was confirmed by the history and examination findings

of (i) local pain (ii) increased pain on palpation over the lateral epicondyle / musculotendinous junction of the common extensor muscle group. (iii) increased pain on resisted extension of the radiocarpal joint.

A neurological cervical spine examination was also carried out to rule out co-existing pathology.

Although a decision was made not to exclude patients who were currently using medications with analgesic properties, including non steroidal anti-inflammatories, subjects were asked to refrain from taking these on the day of scanning. However none of the patients recruited reported the use of any analgesic medication on a regular basis.

Table 5.1 Patient demographics

	Patient A	Patient B	Patient C
Age / Sex	32 / M	41 / M	47 / M
Affected arm	Left	Right	Right
Duration of symptoms (months)	7	4	6

5.2.3 Statistical Analysis

Whilst single subject data can yield interesting findings, extrapolation of findings to the wider population is limited. SPM software package (Wellcome Department of Cognitive Neurology, London, UK) allows for conjunction analysis (Price and Friston, 1999). A Conjunction model is less stringent than a Random Effects model and essentially seeks consistent activations (not necessarily to the same magnitude as random effects) in small samples.

Data collected in this experiment were therefore analysed using the SPM software package, implementing the same design and parameters employed in the previous study; the intention being to undertake a conjunction analysis of the three subjects.

UNIX workstations processed and analysed the data using SPM (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab environment (Mathsworks, Sherborn, MA) (Friston et al, 1995). The scans from each subject were realigned using the first image as a reference. Following realignment, all images were transformed into a standard stereotaxic space (MNI).

The data were then smoothed using a 6 mm isotropic FWHM gaussian kernel. Statistical comparisons between the conditions were performed on a voxel-by-voxel basis using t statistics, generating SPM(t) maps subsequently transformed to the unit distribution SPM(Z) maps. Each regressor used in the GLM was obtained by convolution of an ideal box-car response with a canonical haemodynamic response function (Friston et al., 1995). Statistical significance was estimated voxel-by-voxel with probability criterion of $P<0.05$ corrected for multiple comparisons. Within each region of statistical significance, local maxima of signal increase were determined (the voxel of maximum significance). The anatomical localisation of the local maxima was determined in MNI space.

5.3 Psychophysics Results

Pain intensity – peak visual analogue scale (VAS) score

Mean peak VAS scores for patients A, B & C respectively were ID: 7.1 (.7), 5.2 (1.3) and 3.9 (.48) and for MS: 6.9 (.89), 6.7 (1.1) and 3.1 (.10)

Mean peak unpleasantness scores for patients A, B & C respectively were ID: 6.2 (.73), 5.1 (1.5) and 3.4(.34) and MS; 5.8 (.63), 6.7 (1.1) and 3.0 (.05).

Table 5.2 Summary of psychophysics data

	Patient A		Patient B		Patient C	
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
Peak intensity						
ID	7.1	.70	5.2	1.3	3.9	.48
MS	6.9	.89	6.7	1.1	3.1	.10
Unpleasantness						
ID	6.2	.73	5.1	1.5	3.4	.34
MS	5.8	.63	6.7	1.1	3.0	.05

5.4 FMRI results

Although the analysis intention was to undertake a conjunction analysis on the three subjects; as can be seen from the psychophysical data, Subject C reported very low pain VAS scores therefore the individual analysis reports are reported here.

5.4.1 Subject A (see table 5.4)

Similarities

Ipsilateral activation was observed during both muscle and cutaneous conditions in contralateral thalamus and cerebellum.

Bilateral activation of the hippocampus / amygdala complex was also present in both conditions.

Differences

Muscle

Activation observed during muscle but not cutaneous pain included contralateral anterior and posterior insular cortices and ventrolateral prefrontal cortex; ipsilateral SII; bilateral activation was also observed in the putamen.

Cutaneous

Activation observed during cutaneous but not muscle pain included bilateral pMCC and MPFC.

5.4.2 Patient B (see table 5.5)

Similarities

Bilateral activation was observed during both conditions in anterior insular cortex, cerebellum and MPFC. Contralateral thalamus and SII activation was also observed in both.

Hippocampus activation was observed in both conditions; this was ipsilateral during muscle and contralateral during cutaneous pain.

Differences

Muscle

Bilateral posterior insula activation was seen during muscle but not cutaneous pain; as was ipsilateral MI, SI, putamen and caudate nucleus and contralateral amygdala and PCC activation.

Cutaneous

No differences observed

5.4.3 Patient C (see table 5.6)

Similarities

Ipsilateral SII, hippocampus, posterior insula and bilateral cerebellum activation was present during both pain conditions.

Differences

Muscle

Bilateral perigenual ACC and ipsilateral SI activation was seen during muscle but not cutaneous pain;

Posterior insula and hippocampus activation was also bilateral compared to ipsilateral in cutaneous

Cutaneous

Ipsilateral thalamus, MI and VLPFC activation was observed in cutaneous but not muscle pain condition.

Table 5.4 Areas of activation during muscle and cutaneous pain conditions

Patient A

Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x,y,z	Z score
ACC – pMCC	I			I	5, -6, 44	5.55
	C			C	-4, -12, 40	4.38
Anterior insula	C	-39, 22, 0	6.42	C		
Posterior insula	C	-43, -8, 8	5.89	C		
Thalamus	C	-4, -10, 4	5.23	C	-10, -16, 16	6.48
	I			I		
SII	I	54, -38, 32	5.32	I		
	C			C		
Cerebellum	I	13, -43, -18	5.04	I		
	C	-24, -43, -24	6.62	C	-13, -43, -22	6.72
putamen	I	30, 4, 0	4.92	I		
	C	-31, 4, 2	5.12	C		
Hippocampus	I	27, -10. -20	6.02	I	27, -15, -16	5.44
	C	-26, -36, -10	5.38	C		
Amygdala	I	26, -6, -20	5.74	I	27, 0, -23	4.19
	C	-26, 2, -17	5.43	C	-26, -12, -14	5.27
VLPFC	C	-40, 42, -4	6.59	C		
MPFC				I	2, 50, 0	4.44
				C	-3, 42, -13	4.91

Figure 5.0 Example of hippocampus activation observed during both muscle and cutaneous pain in patient A.

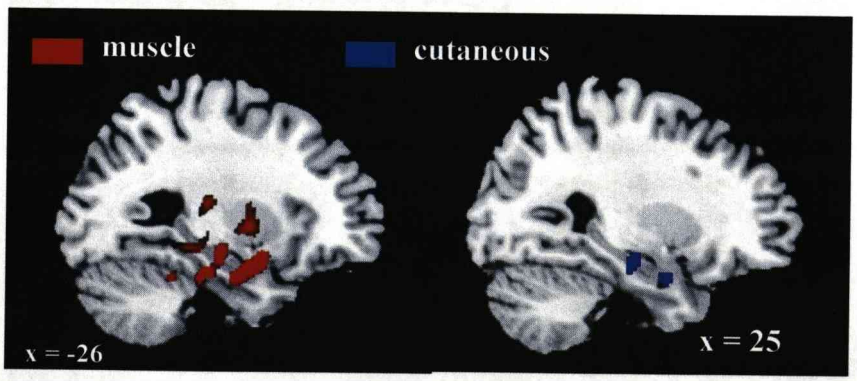


Table 5.6 Areas of activation during muscle and cutaneous pain conditions

Patient B

Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x,y,z	Z score
ACC – pMCC	I	-4, 10, 43	4.67	I		
	C	5, 23, 24	5.43	C		
PCC	I	3, -32, 50	6.01	I		
	C			C		
Anterior insula	I	-33, 16, -1	4.89	I	-38, 1, -4	5.35
	C	44, 9, -5	5.11	C	44, 13, -3	5.84
Posterior insula	I	-47, -7, 3	5.26	I		
	C	42, -8, -2	4.98	C		
Thalamus	I			I	-3, -13, 4	4.72
	C	4, -8, 6	5.33	C	5, -15, 4	4.34
MI	I	-5, -12, 68	6.27	I		
SI	I	-45, -26, 59	5.42	I		
SII	I	-45, -35, 24	5.43	I	58, -21, 31	6.23
	C	54, -35, 24	4.88	C		
Pallidum	C			C		
Cerebellum	I	-20, -53, -17	6.04	I	-27, -73,-23	5.21
	C	26, -54, -47	6.14	C	21, -86, -23	4.34
Putamen	I	-25, 10, -2	4.32	I		
Caudate nucleus	I	-20, 16, 14	5.37	I		
Hippocampus	I	-29, -22, -27	5.41	I		5.51
	C			C	24, -26, -8	
Amygdala	I			I		
	C	29, 0, -30	4.33	C		
MPFC	I	4, 43, -10	4.08	I	-1, 42, -12	5.47
	C	-6, 52, -13	5.11	C	5, 42, -12	4.88

Figure 5.0.1 Example of hippocampus activation observed during both muscle and cutaneous pain in patient C.

Note activation is ipsilateral in muscle condition and contralateral in cutaneous condition

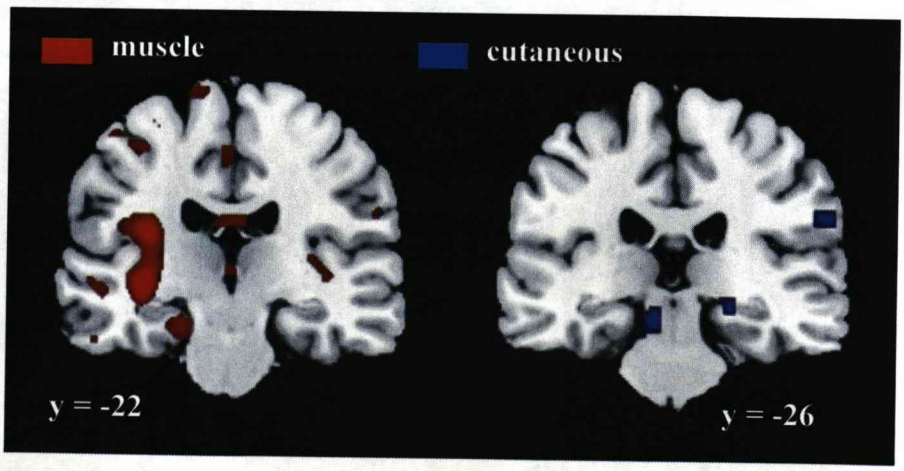
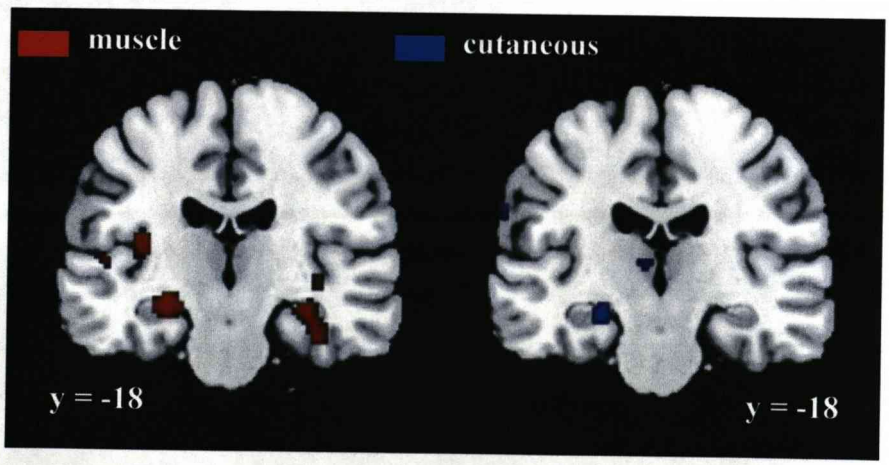


Table 5.7 Areas of activation during muscle and cutaneous pain conditions Patient C

Brain region	Muscle			Cutaneous		
	Side	MNI co-ordinates x, y, z	Z score	Side	MNI co-ordinates x,y,z	Z score
ACC perigenual	I	5, 39, -3	5.44	I		
	C	-3, 40, -3	5.67	C		
Anterior insula	I	-39, 7, 11	6.55	I		
	C			C		
Posterior insula	I	-41, -14, 8	5.98	I	-44, 0, 0	6.22
	C	38, -11, 8	6.35	C		
Thalamus	I			I	-5, -19, 8	5.27
MI	I			I	-6, -15, 77	5.76
SI	I	-19, -41, 73	6.28	I		
SII	I	-49, -29, 29	5.44	I	-49, -29, 29	6.5
Cerebellum	I	28, -78, -37	5.70	I	29, -72, -35	6.78
	C	-48, -54, -37	5.31	C	-47, -52, -34	6.48
Hippocampus	I	34, -18, -15	6.41	I	-26, -17, -13	6.56
	C	-25, -21, -12	7.44	C		
VLPFC	I			I	-44, 34, 0	5.49

Figure 5.0.2 Example of hippocampus activation observed during both muscle and cutaneous pain in patient C.

Note activation is bilateral in muscle condition and ipsilateral in cutaneous condition



5.5 Summary of findings after hypertonic saline injection.

Taken together the three patients appear to show many similarities to those observed in the healthy volunteer group. Additional activation however was also observed in the individual analysis of the patients which was not seen in the healthy volunteer group analysis.

In summary, during the first experiment (injection of HS), areas of the pain matrix were activated including ACC, MI, SI, SII, anterior and posterior insular cortices and cerebellum, suggesting similarity to the healthy volunteer population.

The findings of preponderance towards bilateral activation of so called 'pain matrix' areas during muscle pain as opposed to an ipsilateral or contralateral tendency in cutaneous pain in the healthy volunteers also appears to be mirrored in the patient sample. Basal ganglia activation was also observed in two of three patients during muscle but not cutaneous pain. Again this appears to reflect the findings of the healthy volunteer group, however as the small numbers do not allow for a direct comparison caution must be taken in drawing comparisons.

The co-ordinates of peak activation in SII also appear to be more posterior in the three patients which may suggest a somatotopic shift within the parietal operculum in these particular patients.

The observation of activation in the hippocampus and amygdala in all three patients during both muscle and cutaneous pain (which was not apparent in either condition with the healthy volunteer group) may suggest greater engagement of the hippocampal / amygdala complex in patients, irrespective of whether the pain originates in cutaneous or muscle tissue.

5.6 Methods: Experiment Two

5.6.1 Subjects

Two of the patient subjects from study one also took part in study two (Patients A and C); this was undertaken on a separate occasion from the first scan.

5.6.2 Experimental procedures

A simple pressure algometer was constructed as described by Johnson and Watson (1997), who have shown reliability of this method for repeated pain pressure thresholds. The device consists of a closed low friction syringe with a rubber-tipped probe at the end, the area of which was ~ $1cm^2$; allowing consistent application of predetermined pressure thresholds.
The scale on the syringe may be tested with a pressure algometer to determine actual pressures involved.

Three pressure thresholds were determined; P1 = mild pain, P3 = touch, no pain, P3 = moderate pain

Testing was carried out pre scan to determine the pressures required to produce mild pain (P1, VAS = 3 - 4) and touch (P2, VAS = 0) on both the affected and unaffected arms

Pressure required P1 for the unaffected arm was then tested on the affected arm; if resulting pain was (i) greater than 7 on a VAS and (ii) acceptable to patient then this was deemed P3 for the affected side. P3 on the unaffected arm was then determined by determining pressure required to produce the same VAS as P3 on the affected arm.

Pressure thresholds were retested immediately post scan to ensure background clinical pain had not been exacerbated by the procedures.

5.6.3 fMRI design

Stimuli were delivered manually in blocks of 12 seconds at one of the pre-determined pressures as above in response to a visual cue to the investigator. Subjects were informed that they would be receiving pressure stimuli of a variety of magnitudes throughout the scanning period which may or may not be painful; they were not informed how many variations there were in pressure and were blinded to the order of pressure. Subjects also wore a black-out eye shield to ensure they received no visual cues from the colour cues that were displayed to the investigator to indicate level of pressure application.

5.6.4 Data acquisition

MR images were acquired using a 3T Trio MR Scanner (Siemens, Erlangen, Germany) using a BOLD (blood oxygen level dependent) sensitive T_2 * -weighted multislice gradient echo-planar imaging (EPI) sequence (echo time, 35 ms; repetition time, 3 s; flip angle, 80°; field of view, 224mm; slice thickness, 3.5 mm). Thirty contiguous axial slices were prescribed covering the whole brain. A total of 240 EPI volumes were collected.

5.6.5 Analysis of Imaging Data

After converting scanner data from Dicom utilising MRIConvert v 2.0 (Smith 2006) the resulting FSL compatible Nifti 4d images were loaded into FSLview and observed in movie mode to look for any obvious artefacts or excess motion. Pre-processing steps were applied to each functional dataset: spatial smoothing (Gaussian kernel, full width at half-maximum: 5 mm), motion correction and non-linear high-pass temporal filtering (sigma: 15 s).

A general linear model (GLM) was applied on a voxel by voxel basis to these data (Worsley and Friston, 1995) using FILM (FMRIB's improved linear model) (Woolrich et al., 2001) to model blood-oxygen-level-dependent (BOLD) signal

intensity changes in response to painful stimuli. The subject level statistical images were registered into MNI (Montreal Neurological Institute) standard space using FLIRT (Jenkinson and Smith, 2001).

A single regressor for each level of pressure was modelled; each regressor constructed by convolving a boxcar function with a gamma haemodynamic response function. Voxel-wise parameter estimates (PEs) were derived for each regressor and for each subject a statistical image was calculated for each EV. Appropriate contrasts were modelled to enable comparisons in activation between the different pressure thresholds.

5.7 Psychophysics results

Application of P1 (mild pain) determined in the unaffected arm, to the affected arm in both subjects resulted in a moderate to high pain (>7) on a VAS score. This was therefore implemented as P3 in the affected arm. Pressure required to produce a moderate to high VAS on the unaffected arm was considerably higher.

5.8 fMRI results

5.8.1 Pressure pain

Both patients were interviewed immediately after the end of scanning to assess whether a summation effect occurred during the pressure pain paradigm in either the affected or unaffected side; both denied any significant increase in background pain.

Both subjects demonstrated activation distributed throughout various regions of the brain during pressure pain on both affected and unaffected areas. Areas of increased signal included bilateral ACC, posterior and anterior insular cortices, MI, SI, thalamus, putamen and cerebellum.

5.8.2 Matched pressure

A contrast was carried out between P1 on the unaffected arm and P3 on the affected arm i.e. amount of pressure delivered was similar but pain scores were greater on the affected side. No activation survived the P1 > P3 contrast (Z> 2.3, cluster size minimum 10).

Widespread activation was apparent in the P3 > P1 contrast; some co-ordinates are presented in table 5.8 to indicate areas of activity.

Figures 5.1 and 5.2 demonstrate the widely distributed activation patterns and are included for illustrative purposes.

Table 5.8
Areas of brain activation during pressure matching affected (P3) > unaffected (P1)

Brain region		Patient A		Patient C
	Side	MNI co-ordinates x,y,z	Side	MNI co-ordinates x,y,z
ACC	I		I	6, 34, 10
	C	2, 22, 28	C	
Anterior insula	I	-22, 32, 4	I	34, 24, 0
Thalamus	I	-12, -14, 6	I	
	C		C	-18, -26, 2
MI	I	-20, -26, 60	I	
	C		C	
Putamen	I	-20, 6, -2	I	16, 14, -6
	C	28, -2, -2	C	
caudate	I	-16, 16, 2	I	
Precuneus	I		I	
	C		C	-4, -68. 44
Amygdala	C		C	-18, -14, -18
Hippocampus/ parahippocampus	I		I	20, -28, -8
	C	22, -8, -26	C	
DLPFC	I	-36, 32, 32	I	36, 30, 24
	C		C	
MPFC / orbitofrontal	I	-10, 42, -18	I	14, 20, -8

Fig 5.1 Patient A – activation observed during matched pressure (affected > unaffected)

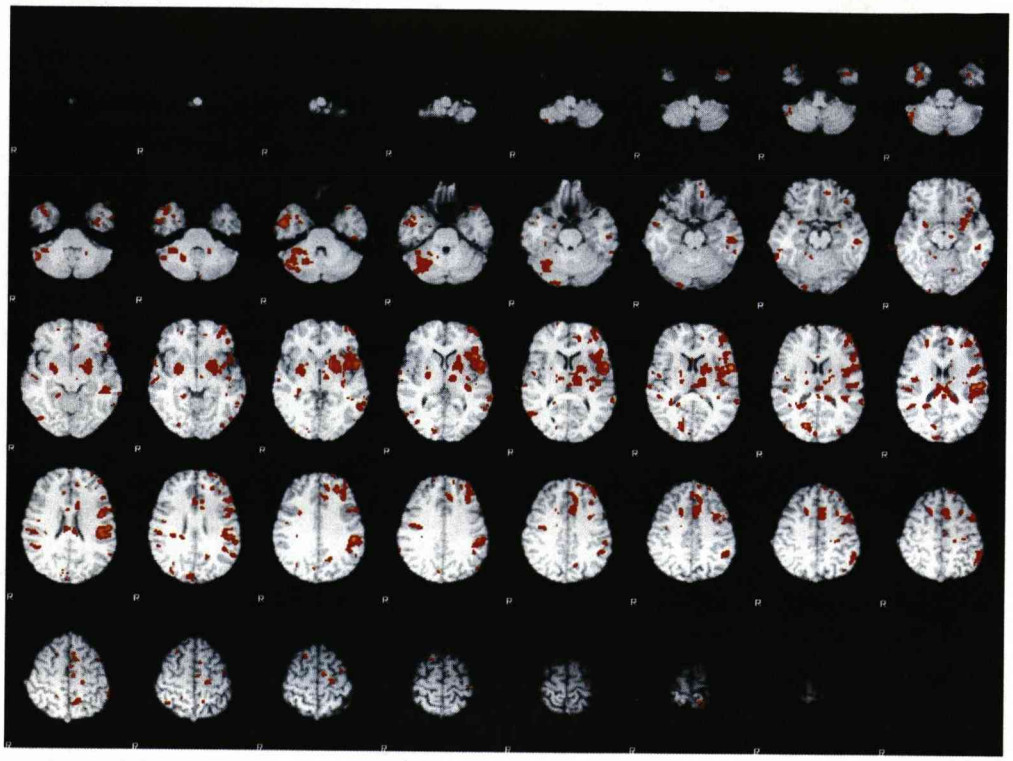
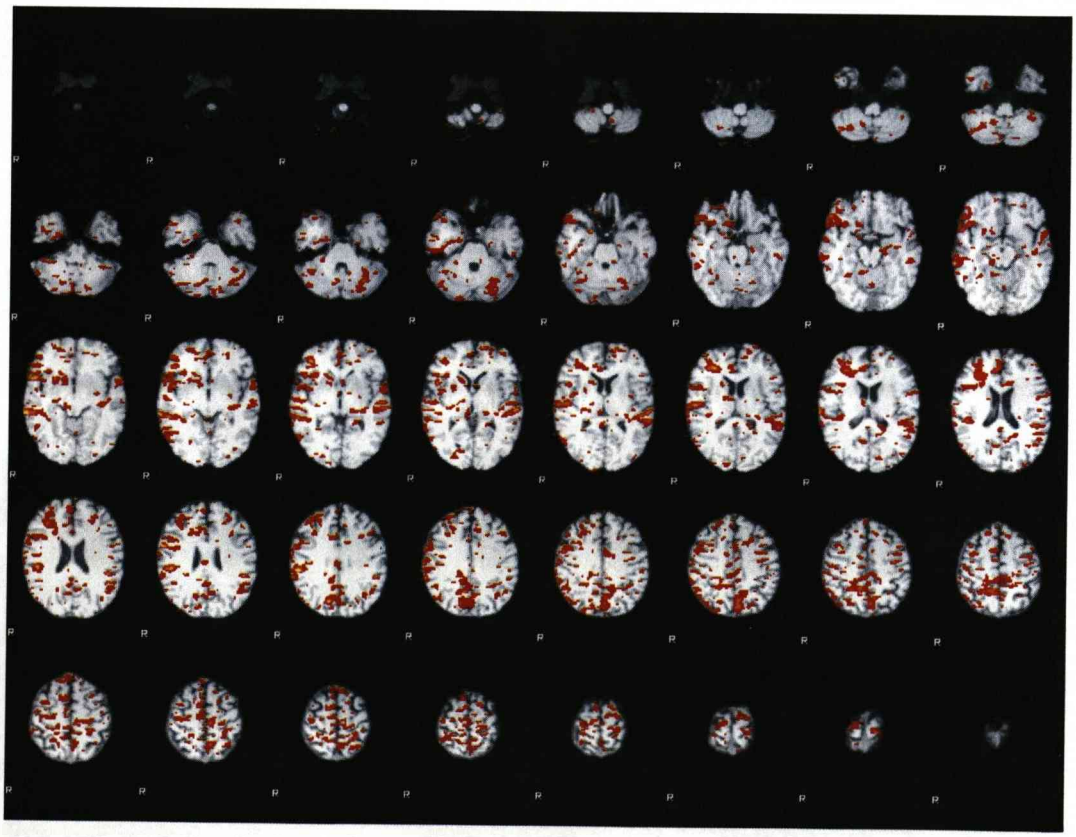


Fig 5.2 Patient C: activation observed during matched pressure (affected > unaffected)



5.8.3 Innocuous pressure

Marked widespread activation was observed during innocuous pressure to the affected arm. A further contrast was therefore investigated; innocuous pressure in affected arm > innocuous pressure in the unaffected arm.

Results of this contrast are presented in table 5.9 and figure 5.3 and 5.4
No activation survived threshold (Z> 2.3, cluster size minimum 10) in the opposite contrast.

Table 5.9 Brain regions showing increases in BOLD signal during innocuous pressure on the affected side compared to the unaffected side

Brain region		Patient A		Patient C
	Side	MNI co-ordinates x,y,z	Side	MNI co-ordinates x,y,z
ACC	I	-8, -2, 40	I	14, 0, 40
	C	12, 22, 32	C	10, 2 , 42
PCC	I	-8, -32, 44	I	
Anterior insula	I	-44, 14, -6	I	
Posterior insula	I	-8, -18, -4	I	
	C		C	-16, -28, 6
MI	I	-18, -24, 60	I	34, -20, 66
SI	I	-36, 42, 60	I	32. -40, 68
	C	36, -40, 64	C	
Amygdala	I		I	20, -6, -16
	C	22, -2, -6	C	-22, -12, -16
Hippocampus / parahippocampus	I		I	22, -18, -6
	C		C	-16, -12, -22
DLPFC	I	-34, 34, 24	I	
	C		C	-38, 24, 38
VLPFC	C		C	-42, 56, 2
MPFC / orbitofrontal			C	-16, 62, 6

Figure 5.3 Patient A Areas of activation during innocuous touch (affected > unaffected)

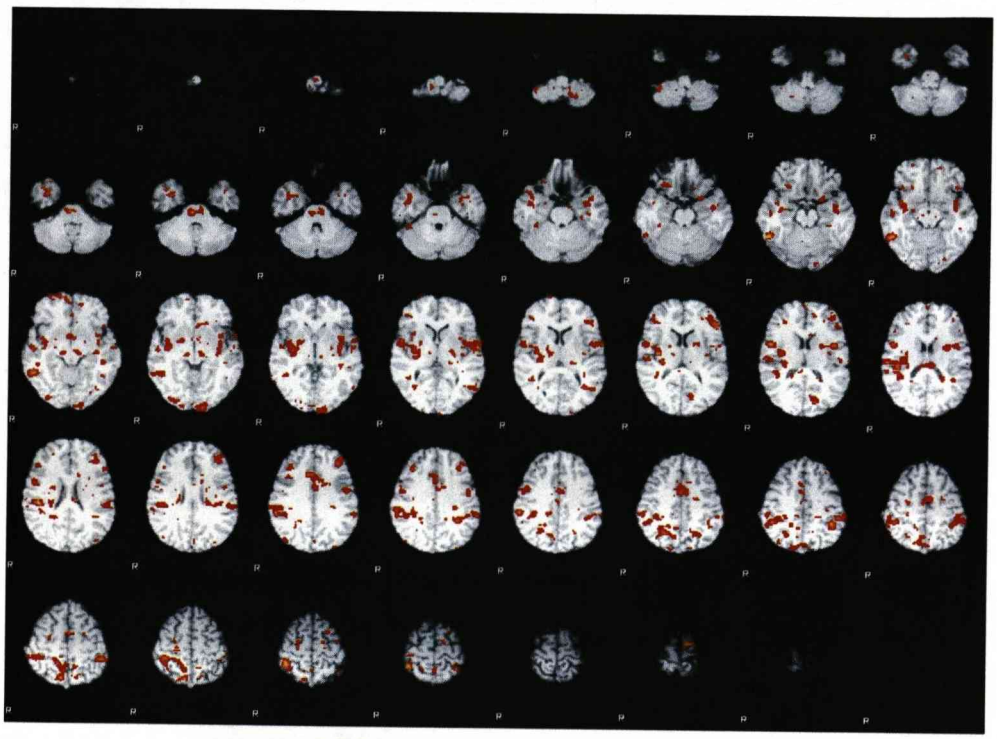
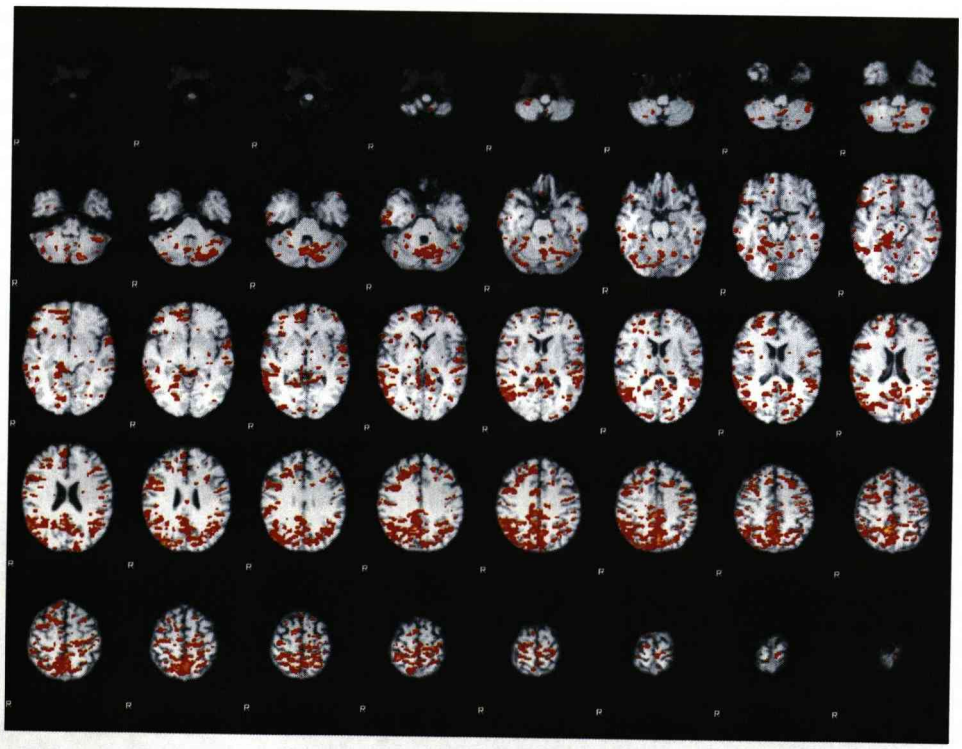


Figure 5.4 Patient C Areas of activation during innocuous touch (affected > unaffected)



5.9 Summary of pressure pain findings

During pressure pain on both the affected and unaffected arms in two patients with tennis elbow, widespread brain activation was observed in areas consistent with previous reports of pressure pain.

When stimulus intensity but not pain intensity was matched, greater activation was observed when the stimulus was applied to the affected arm than when applied to the unaffected arm.

Finally during innocuous (i.e. non pain provoking) stimulus application to the affected arm, widely distributed activation was again observed in a number of brain regions known to be active during a noxious stimulus.

5.10 Chapter summary

The over-riding caveat when interpreting the results of the patient data is of course that any interpretation or attempts at extrapolation are limited due to the small number of subjects. Nevertheless the activation patterns observed in the patients make for interesting discussion.

Taken together the three patients appear to show many similarities but also some key differences in brain activation patterns than those observed in the healthy volunteers.

In summary, during the first experiment (injection of HS), areas of the pain matrix were activated including ACC, MI, SI, anterior and posterior insular cortices and cerebellum, demonstrating similarity to the healthy volunteer population.

Preponderance towards bilateral activation of so called 'pain matrix' areas during muscle pain compared to an ipsilateral or contralateral tendency in cutaneous pain was observed in the healthy volunteer group described in the previous chapter. This finding does also appear to be mirrored in the small patient sample reported here although a direct comparison was not carried out therefore again caution must be applied in drawing direct comparisons.

Basal ganglia activation was also observed in two of three patients during muscle but not cutaneous pain which again would appear to reflect the findings of the healthy volunteer group.

Activation in the hippocampus and amygdala in all three patients during both muscle and cutaneous pain was present when compared to rest on an individual analysis basis. This may suggest greater engagement of the hippocampal complex in patients as this finding was not observed in the healthy volunteer group analysis during either cutaneous or muscle pain. Further investigation of a larger number of patients would allow a direct comparison to be undertaken and therefore further exploration of this interesting finding.

Chapter 6
Discussion

This thesis describes four experiments; one each in chapter three and four and two in chapter five; the first two carried out with separate groups of healthy volunteers, the latter two with two / three patients who had a diagnosis of unilateral lateral epicondylitis (tennis elbow).

This final discussion chapter begins with a summary of the findings, followed by a review of associated methodological issues and then further discussion of each experiment in turn.

The chapter concludes with a discussion regarding the implications of the findings of all three experiments and makes recommendation for future investigations.

6.1 Psychophysical properties of deep and superficial pain

The first experiment described in this thesis was an investigation of the psychophysical properties of pain arising from superficial and deep tissue, induced by the same method; in this case hypertonic saline. Two locations of superficial tissue were investigated – intradermal and subcutaneous, whilst one deep tissue was investigated; extensor digitorum muscle of the forearm.

Summary of results from this study

Peak intensity, as measured by visual analogue scale, of hypertonic saline induced pain in intradermal or muscle tissue in healthy volunteers was of similar magnitude within and between sessions. The selected injection volumes reported produced peak intensity scores that were very similar within subjects, irrespective of whether the injection was ID or MS. In contrast, SC injections gave VAS scores that were less comparable than those reported after ID or MS injections.

It is likely that during ID and MS injections the nociceptors (i.e., free nerve endings) that abound in the skin and muscle are directly stimulated by the injectate. By contrast, the SC injectate can only reach the dermal and intradermal nociceptors by diffusion. The subcutis itself is almost devoid of free somatic nerve endings although autonomic fibres and mechanoreceptors (e.g., Pacinian corpuscles) are found. The present results reflect those reported in a rat study investigating the hyperalgesic effects of prostaglandin E_2 and bradykinin (Khasar et al., (1993); After intradermal and subcutaneous injection of these inflammatory mediators, the investigators observed dose dependent lowering of paw withdrawal thresholds after ID but not SC injection. The authors suggest the lack of effect of the SC injection may reflect the inability of the injectate to reach its receptor sites in the terminals of the primary afferent nociceptors.

The findings reported here indicate that subcutaneous injection of an algesic substance is less reliable that intradermal injection.

The time to reach peak intensity after intradermal injection was less than it was for subcutaneous and muscle and therefore predictably area under the curve for the initial 60s VAS data was also greater after intradermal injection. However, no difference was reported between 'total pain' intensity, as measured over the

ensuing 210 seconds, between intradermal and muscle pain, although a significant difference between intradermal and subcutaneous conditions was found.

Unpleasantness scores were also similar between muscle and intradermal pain and showed a strong positive linear relationship with peak intensity scores.

Pain arising from muscle and intradermal tissue could be reliably differentiated through selection of sensory descriptors from the SF McGill Pain Questionnaire. During muscle pain words most frequently selected and rated higher were 'gnawing', 'aching' 'heavy' and 'cramping' whilst during intradermal pain 'hot/burning', 'stabbing' and 'sharp' were selected more frequently and scored higher than both subcutaneous and muscle. Subcutaneous pain resulted in no descriptor being selected either frequently or scoring higher than either intradermal or muscle pain.

Methodological issues

The use of hypertonic saline injections over other methodologies was based on practicalities dictated by the need for suitability in MRI environment, consistency in evoked pain, controllability, repeatability, a relatively short duration of pain elicited, and acceptability in a patient population. The key benefits in using hypertonic saline were considered to be (i) the same stimulus was used for both cutaneous and muscle pain ensuring, at least in theory, a similar mode of activation of peripheral nociceptors, (ii) localisation of needles was the same, and the subject was not distracted or confused by two different pain eliciting modes (e.g., laser or contact heat in the skin and injections to the muscle as employed in a number of previously reported studies) (iii) the injections could be carried out in the same setting, (iv) the magnitude of provoked pain could be easily adjusted by the volume of the injectate. The author also predicted that the time course following each injection would be comparable, and that the subjects were able to rate the intensity and unpleasantness of provoked pain separately. As discussed above, the key similarities between ID and IM injections were evident, providing support for the chosen method and allowing it to be implemented in the next stage of the study.

Some limitations of the method however should be considered. Referred pain was commonly seen during muscle pain but not in either of the cutaneous pain conditions. This may be seen as a weakness in terms of comparison as extent of referred pain may contribute to the intensity or affective quality of a painful stimulus. The findings however are in agreement with the literature in that referred pain is a phenomenon associated with pain arising from muscle but not cutaneous tissue (Graven-Nielsen et al., 1997; Arendt-Nielsen., 1996). Mechanisms underlying referred pain are not fully understood but are suggested to involve convergence of inputs into the spinal cord by separate sets of afferents, as well as aspects of central hyperexcitability (Hoheisel et al., 1993). Referred pain from a muscle or cutaneous injection has recently been shown to be reflected in an increased activation in somatosensory cortex (which is somatotopically oriented), cerebellum and mid cingulate regions of the brain (Macefield et al., 2007).

A further potential confounding factor in investigating repeated pain within the same session is that of habituation. Attention was paid to the time intervals between injections which were selected to ensure that temporal summation did not occur (Graven-Nielsen et al., 1997) but it was impossible to predict from the small pilot study whether or not habituation would occur. Although intensity data showed no statistically significant difference within session for muscle and intradermal pain, visual inspection of the plotted data suggests a trend for a decrease in peak VAS in all conditions during the second injection within both sessions. These findings however provided further guidance with regards to timings and number of needles required for the fMRI experiment.

A further criticism that may be levelled is that despite a great deal of attention to methodology in the attempt to control as many confounding variables as possible, actual pain values reported by individuals showed a wide range suggesting that the study design may be less than optimal. Furthermore, inspection of individual VAS scores suggested that failure of the technique with regards to muscle pain may have occurred in up to four injections, although not in the same subject. The latter issue in particular provided further insight into the need to perform the fMRI experiment with meticulous attention to the details of pain provocation.

The present results add to the limited literature that exists of direct comparisons of pain induced in the two tissue types employing the same method of induction (Witting et al., 2001).

It is worth mentioning again at this point, that in addition to the investigation of the similarities / differences between pain induced in two tissues types using the same method of induction, the experiment was predicted to produce a reliable model to take into a scanning environment. Indeed, the results helped to guide the technique of injection, selection of injectate volume for each tissue type, and providing a predictable time course for scanning; adding to the intention of the investigator to have a methodologically sound way of invoking pain of sufficient intensity but limited duration

Further discussion

Unpleasantness of a painful stimulus has been shown to be dependant on stimulus modality (Price et al., 1983; Rainville et al., 1992; Svensson et al., 1997) and temporal aspects (Chen and Treede, 1985; Miron et al., 1989). Although the temporal aspects between muscle and intradermal pain showed some variation in the present study; both gave rise to a tonic rather than phasic pain and therefore may be considered comparable.

Experiment one produced pain of comparable intensity, duration and unpleasantness in two distinct tissue types; differences reported in sensory descriptors therefore may reasonably be attributed to differences in the perceived quality of pain arising from either cutaneous or muscle pain independent of time and type of stimulus.

It was hypothesised that subjects would select affective words more frequently to describe the pain induced in the muscle compared to that induced in the subcutaneous or cutaneous tissue. Previous reports have suggested that experimentally induced muscle pain may result in a relatively larger activation of affective mechanisms (Svensson et al 1997) and more closely mimic chronic pain (Rainville et al., 1992; Arendt-Nielsen et al., 1997). The findings here of lack of selection of affective words from the SF McGill pain questionnaire however do not lend support for the hypothesis.

The lack of selection of affective words may reflect the study population of healthy volunteers as opposed to a patient population particularly those with chronic pain; participants were aware that induced pain, even if intense and unpleasant, would be of relatively short duration. A further consideration is whether the SF McGill is sensitive enough to detect any difference in the affective response to muscle and cutaneous pain. Although previous studies have shown this to be the case (Svensson et al., 1997a; Rainville et al., 1992), again these studies did not compare the same method of induction therefore differences may have been related to type or temporal aspect of stimulus rather than tissue type. The only study to date which has used the same stimulus compared intradermal and intramuscular capsaicin injection (Witting et al., 2001). Whilst that group did use the McGill Pain Questionnaire, it was the long form version therefore direct comparisons would be difficult to make. In addition the authors provided only a brief description of the words selected and did not separate these into sensory and affective.

Previous reports also suggest pain arising from deep tissue is found to be more unpleasant than that from superficial tissue. Relative unpleasantness of contact heat and cutaneous electrical pain was found to be less than both ischaemic exercise and cold-pressor induced pain (Rainville et al., 1992) whilst Svensson et al (1997) reported greater relative unpleasantness during experimentally induced muscle compared to cutaneous pain. Results of the experiment presented here are again contrary to both of these previous findings in that no difference was found in unpleasantness ratings between cutaneous and muscle pain. Possible reasons for the differences are (i) differences in the temporal aspects of the stimulus presentation and pain induced; in Rainville and colleagues' study (1992) the two stimuli shown to be relatively more unpleasant (ischaemic exercise and 'cold-pressor' were of a tonic, that is longer duration, whilst the less unpleasant stimuli (contact heat and cutaneous electrical stimulation) consisted of short lasting phasic pain. A number of studies report on both intensity and relative unpleasantness of a painful stimulus without systematically evaluating the temporal relationship. However those investigating short duration (typically < 5s) have tended to report unpleasantness ratings that are lower than intensity (Price et al 1983, Harkins et al., 1986; Duncan et al., 1989).

Conversely those investigating more tonic pain appear to suggest that affective or unpleasantness ratings are equal or greater than perceived intensity (Maixner et al., 1990; Chen et al., 1989).

It should also be noted that both studies employed different modalities of pain induction and differences observed in unpleasantness ratings may reflect the relative affective response to different types of stimulus rather than tissue involved. As previously stated, cutaneous and muscle pain in the experiment described here was induced by the same modality (hypertonic saline) and resulted in pain of similar duration, albeit the onset slope was greater in intradermal whilst the offset was slower in muscle. One of the aims of the first experiment was to investigate the psychophysical properties of muscle and cutaneous pain when the temporal aspects and induction methods were closely matched which did not allow for further exploration of relative unpleasantness / intensity differences at varying stimulus intensities. Although unpleasantness was found to correlate with peak intensity, as only one unpleasantness rating was taken it was also not possible to determine if the relative unpleasantness varied over time. It was not feasible to obtain simultaneous ratings of intensity and unpleasantness; however, it would have been possible to obtain more frequent unpleasantness ratings which would allow changes in unpleasantness to be evaluated as a function of time.

The presented results however are consistent with the hypothesis that when subjected to a similar peripheral stimulus, healthy subjects perceive pain arising from skin and muscle tissues as qualitatively different in that SF McGill sensory descriptors vary significantly.

These results provided the foundation for the next hypothesis; that neural correlates of each pain are likely to show differences and also the methodology for the next investigation. It was hypothesised that such differences are likely to be found in the activation patterns of structures that are generically referred to as the pain matrix.

6.3 Neural correlates of deep and superficial pain

Chapter four describes the main fMRI experiment in which eighteen healthy volunteers were exposed to both cutaneous and muscle pain of similar intensity, duration and unpleasantness and arising from the same type of stimulus. The author will discuss here in what way the differences observed in brain activation patterns may be attributed to differences in the sensory, affective, cognitive and motivational qualities that are generated by noxious stimulation of the two separate tissue types.

Summary of fMRI results in healthy volunteers

Both cutaneous and muscle pain were associated with an increase in BOLD signal in bilateral primary sensory and motor cortices (SI and MI) and anterior and posterior insula cortices. Bilateral anterior cingulate signal increase was also present with both conditions demonstrating separate peaks of activation in the pMCC and aMCC. In a number of brain regions bilateral signal increase was observed during muscle whilst during cutaneous pain this was only contaralateral (secondary sensory cortex (SII), the cingulate motor area (CMA), orbito-frontal cortex and the putamen) or ipsilateral (DLPFC, VLPFC, PCC). Areas where signal increase occurred only in muscle pain were bilateral thalamus and MPFC, and contralateral perigenual ACC, caudate nucleus and cerebellum.

During attention to / rating of intensity increases in BOLD signal were seen bilaterally in ACC and SMA and ipsilateral in MI and SI in both conditions. Bilateral putamen activation was also observed during attention to intensity of muscle but not cutaneous pain. Bilateral ACC and SMA and ipsilateral MI and SI activation showed a positive linear variation with intensity ratings; no differences were seen between the two conditions in terms of intensity co-variance.

During attention to unpleasantness an increased number of brain regions were activated including bilateral putamen, pallidum and prefrontal cortex. Those areas showing a positive linear relationship with unpleasantness ratings included

bilateral ACC and MI, ipsilateral anterior insula, OFC and cerebellum and contralateral DLPFC during both conditions. Additional areas showing a positive linear relationship during muscle pain only included ipsilateral caudate nucleus and putamen and bilateral VLPFC.

A decrease in BOLD signal that co-varied linearly with unpleasantness scores during attention to unpleasantness in the muscle but not cutaneous condition was also seen in bilateral amygdala and contralateral cerebellum and posterior insula.

Methodological issues

Due to the nature of the pain induced the duration of scanning paradigm was long; approximately 36 minutes. Potential problems associated with longer paradigms include increased tendency for motion artefacts, scanner drift, changes in level of attention / concentration from the subjects, lack of acceptability of procedure for subjects.
However initial scanning was undertaken, incorporating a number of paradigms, on five subjects to assess the affect of and to minimise these issues. These initial results and subjects were evaluated for reproducibility, technical variations, tolerability in terms of time, levels of pain induced, and compliance with the task. Motion artefacts were of particular interest given the duration of the scans.

Post scan interviews revealed that the time period was completely acceptable; indeed, most subjects expressed surprise when informed of the total time in the scanner, reporting that 'it felt considerably less'. These interviews also revealed that instructions projected were clear and understandable and that the levels of pain produced, although unpleasant were acceptable in the time frame that they were presented.

Importance of not moving was stressed to subjects and a significant period of time was spent ensuring maximum level of comfort was met, through use of appropriate padding on the scanner bed, prior to commencement of the scan. Each subject's head was restrained by padded side bars on the head coil but due to the length of scan the decision was made not to employ the use of a bite bar. Pre-

processing of the acquired data indicates that these measures were successful with only one subject having to be excluded due to excess motion.

Attentional aspects were managed by frequent changes in projected instructions. Specifically during the SF McGill pain questionnaire words subjects were instructed to touch the VAS button once to acknowledge that the word had been read therefore ensuring attention to each word individually.

Post-scan interviews also revealed that subjects felt that the presence of the investigator in the scan room throughout the period of acquisition of the functional images provided a reassuring presence which reduced anxiety due to the noise and enclosed nature of an MRI scanner. Levels of anxiety however were not formally measured which may be seen as a weakness in the methodology as this could effect the results. Although such data would have been interesting it was not felt necessary as it was considered that any situational anxiety caused by the scanning environment would be similar during both muscle and cutaneous pain.

All subjects were screened by and provided verbal instructions from a single investigator (the author) to ensure consistency of information. The same investigator also inserted the cannulae and subsequently administered the injections thereby also ensuring consistency of technique.
All data reported in experiment two was analysed by the author using the FSL analysis software suite, having undertaken training in the use of FSL. However to ensure no systematic error occurred during data analysis, three subjects' data sets were selected at random and analysed using SPM 2 (Friston 2002) by a senior research fellow with extensive experience of fMRI analysis. The results were then visually inspected to check that results were comparable.

Another limitation perhaps is that data regarding individual psychometrics, for example levels of fear avoidance, catastrophizing were not gathered. The justification for this being that it was expected that if an individual had had high levels of catastrophizing that they would have a heightened response equally to both cutaneous and muscle pain. In retrospect of course one could argue that

given the elements observed in brain activation a stronger level of catastrophizing may have been associated with a stronger response in the muscle pain condition.

Although this question was not a specific aim of the experiment, in view of the findings, this may lead to a future line of investigation. It is also worth noting that subjects were self – selecting, that is they answered an advertisement and were aware that they would be undergoing an experiment whereby pain of moderate to high levels would be induced. It is possible therefore that this self selection would have filtered out extremes with regards to psychometric profile.

It is argued that a particular methodological strength of this experiment was that it sought to undertake a direct comparison between superficial and deep pain induced by the same method and therefore differences observed can not be explained by differences in stimulus type. As previously discussed experimental pain induced by different methods result in different levels of reported unpleasantness (Rainville et al., 2002, Svensson et al., 1997a).

Typically, pain studies also threshold pain at the beginning of a scan and make the assumption that pain levels are consistent throughout. Although extensive laboratory testing of the model indicated a high level of consistency both within and between sessions, a number of trials did not result in pain rated at least three on the visual analogue scale. Whilst this may be seen as a methodological weakness, through gathering psychophysics data in real time, events where pain did not reach 3 on the VAS scale were modelled as events of no interest in the final analysis. Therefore it can be stated with confidence that events included in the 'muscle' and 'cutaneous' conditions were all pain events. The selection of 3 as the inclusion criteria was based on that previously reported (Henderson et al., 2006, 2007; Kupers et al., 2004). Furthermore additional ratings of unpleasantness and SF McGill were also able to be taken into account before pain epochs were included in the final analysis model.

Discussion

A review of the literature undertaken at the beginning of this course of study failed to reveal any brain imaging studies that had adequately undertaken a direct comparison between cutaneous and muscle pain employing the same method of induction. However Henderson and colleagues published in 2006 the results of a study utilising fMRI to investigate 15 healthy volunteers during both HS induced muscle and cutaneous pain; the first group to undertake this direct comparison; the results of which were reviewed in chapter two. Subsequently this group of investigators have also published further studies employing the HS model reporting on gender differences between muscle and cutaneous pain (Henderson et al., 2008), somatotopy for muscle and cutaneous pain in the insula (Henderson et al., 2007) and associated referred pain (Macefield et al., 2007).

Results from the study presented here provide some support for but also asks questions of the findings of Henderson and colleagues in relation to brain processing of muscle and cutaneous pain in healthy volunteers. There were commonalities in findings between those published and the results of the study presented here but crucially also differences, particularly in relation to subcortical structures and perienual ACC. Potential explanations for the differences will be explored below.

The results reported here also add a new dimension by application of the experimental model in admittedly small clinical pain condition; the findings of which again make for interesting discussion, addressed later in this chapter.

Anterior Insula

The finding of Henderson et al., (2006) of ipsilateral anterior insula activation only during the deep pain condition is somewhat surprising and in contrast to the majority of the literature with AI being the most consistently reported region of activation in both experimental (Coghill et al., 1999, Craig et al., 2000, Derbyshire et al., 1997, Porro, 1998 and Tolle et al., 1999) and clinical pain studies (Maihofner et al., 2003, Peyron et al., 2004 and Petrovic et al., 1999 and

Schweinhardt et al 2006). The authors suggest their results may be due to the tonic nature of the stimulus or the type of stimulus. However this would not explain the difference seen in their results compared to those reported here. The reliance of Henderson's group on only a single injection may have given insufficient power to detect signal changes and is an alternative explanation.

Methodological issues or insufficient power may well explain Henderson and colleagues (2006) initial findings as the same group of investigators in two later studies involving a larger group of subjects did this time demonstrate ipsilateral AI activation during both cutaneous and muscle pain. Furthermore reported co-ordinates show that not only is the peak of activation in rostral AI but it is also somatotopically organised (Macefield et al., 2007) in both cutaneous and muscle pain conditions.

The results observed in the healthy volunteer study therefore is in agreement with the majority of the literature in that strong bilateral anterior insula activation was observed during both superficial and deep pain conditions; the peak of activation in the group analysis was more rostral during deep pain. Ipsilateral rostral anterior insula activation also survived the muscle > cutaneous contrast suggesting greater activation during muscle pain compared to cutaneous pain.

The insula receives input from spinothalamically activated posterior thalamic nuclei, has projections to the amygdala (Burton and Jones, 1976, Friedman and Murray, 1986) and is considered part of circuitry related to fear avoidance (Morris et al., 1999). Engagement of the anterior insula has also been reported during other aversive challenges including aversive gustatory stimulation (Zald et al., 1998), fearful vocalizations (Morris et al., 1999), heartbeat detection (Critchley et al., 2004) hunger (Tataranni et al., 1999) and air hunger (Evans et al., 2002).

Encoding of perceived intensity of experimental pain in healthy volunteers is also consistently found in anterior insula (Coghill et al., 1999; Craig et al., 2000; Derbyshire et al., 1997 and Peyron et al., 1999). It has been suggested that as interoception moves from sensation to cognition, initial sensory recognition occurs in the posterior insula then remapped to the right anterior insula via

corticocortical and/or callosal pathways (Kong et al 2006). The second-order representation in the right AI is proposed to subserve subjective feelings (Craig et al., 2000; Craig, 2002); suggesting AI has an important role in cognitive modulation of pain perception (Kong et al., 2006).

In the present study, it was hypothesised therefore that muscle pain, being less familiar and having greater emotional salience than cutaneous pain would provoke a greater response in the rostral anterior insula when compared to cutaneous pain. The finding of ipsilateral rostral anterior insula activation that survived the muscle > cutaneous contrast should be interpreted with caution given the bilateral activation seen during both conditions compared to rest. It is not uncommon within the literature for researchers to present only the results of a contrast when undertaking comparisons of two or more conditions; the anterior insula findings discussed here perhaps highlight the importance, in this type of study, of reporting group activation maps as well as contrasts to facilitate discussion and interpretation of results.

As this study was only undertaken on one arm (left) it is not possible to infer anything from the results regarding laterality of insula activation which may be seen as a weakness in the methodology. However the use of the non dominant arm in the healthy volunteer group was specifically chosen to enable comparison with the planned patient group.

Although the results do indicate that the peak of anterior insula activation during muscle pain is more rostral than that observed during cutaneous pain, when compared to the meta-analysis undertaken by Schweinhardt et al (2006), experimental pain studies. Indeed, although in the group analysis the co-ordinates of peak ipsilateral activation during cutaneous pain fell within the caudal part of the AI (as defined by Schweinhardt et al, 2006), ROI analysis on an individual subject basis revealed distinct areas of activation during both conditions, in both rostral and caudal AI bilaterally.

A possible explanation for this finding may be the tonic nature of stimulation employed in this study in comparison to the brief phasic pain typically employed

in other experimental studies. Pain that is prolonged or inescapable is more aversive (Lumb et al., 2002) therefore it is not entirely surprising that both conditions resulted in strong rostral anterior insula activation. Indeed a positive linear relationship with unpleasantness ratings during both conditions was evident during attention to unpleasantness.

The results therefore suggest that encoding of pain in the rostral AI may be related to the inescapable / aversive nature of prolonged pain rather than supporting the argument that it reflects the difference in nature between the tissues from which the noxious input is arising. A study investigating differences between short lasting muscle and cutaneous pain induced by the same method would facilitate further exploration of the question of whether rostral AI does also encode for pain of tissue type.

Posterior Insula

It has been suggested that posterior insula activation during experimental pain reflects basic sensory aspects of nociceptive input itself as opposed to the subjective experience of the pain (Apkarian et al., 2005, Brooks et al., 2005, Ostrowsky et al., 2002); opinion which is supported by the findings of somatotopic organisation in posterior insula (Brooks et al., 2005 and Craig 1995, Henderson et al., 2007; Macefield et al., 2007, Craig et al, 2000) suggests posterior insula may encode intensity of the nociceptive stimulus rather than the actual perceived pain intensity.

A significant increase in signal was observed bilaterally during both muscle and cutaneous pain in this study with no differences between the two conditions. This result is consistent with opinion from published literature that the posterior insula encodes for pain intensity; as intensity ratings were similar between muscle and cutaneous pain conditions, it was not anticipated that differences would be found in this region.

Prefrontal Cortex

Frontal lobe activity during pain in general is considered to be related to cognitive and attentional processes (Coghill et al 1999, Casey 1999, Peyron et al 1999).
MPFC has also been shown to be involved in negative emotions, response conflict, and detection of unfavourable outcomes, especially in relation to the self (Baliki et al., 2006).

Previous studies suggest that dorsolateral prefrontal cortex (DLPFC), is important for continuous monitoring of the external environment, information processing of short-term memory and directing appropriate and efficient performance in the presence of aversive or distractive stimuli (MacDonald et al., 2000, Bunge et al., 2001; Miller et al., 2001; Fuster, 2001).

Wager et al (2004) suggests that DLPFC has a role in the placebo response, demonstrating increased signal change DLPFC during anticipation of pain relief that correlated with increases in midbrain signal anticipation, which the authors suggest is consistent with their hypothesis that opioid release in the midbrain is triggered by prefrontal mechanisms. Although acknowledging the alternative interpretation; that DLPFC redirects attention away from pain, having been implicated in studies investigating general attentional processes (Peyron et al., 1999) they cite their midbrain findings as further evidence that placebo is opioid mediated and is reflected in DLPFC activity.

Lorenz et al (2003) report that during capsaicin induced heat hyperalgesia, unpleasantness ratings were significantly higher during low compared with high DLPFC activity; the authors suggesting that the DLPFC modulates the unpleasantness of the pain. An inverse relationship was also demonstrated in the correlation of midbrain and medial thalamus activity during left DLPFC activation – the correlation being less strong during left DLPFC activity suggesting the DLPFC has a 'top-down' modulation of midbrain / medial thalamus circuitry.
It is interesting therefore to consider the finding of increased DLPFC activation during muscle pain compared to cutaneous pain in the results reported here. When one also considers the lack of perceived difference in unpleasantness between the two conditions, a possible interpretation is that muscle pain is

initially more unpleasant but DLPFC subsequently mediates the degree of unpleasantness. Unpleasantness ratings were recorded approximately two minutes after onset of pain therefore it is not possible to confirm this although it does make an interesting hypothesis. Future studies collecting serial unpleasantness ratings would be useful to investigate this further.

Lorenz et al., (2003) also report that the relationship of the perigenual ACC activity with unpleasantness was left DLPFC dependent; a positive correlation was present during low left DLPFC activity, whilst a negative correlation was observed during strong left DLPFC activity. The authors suggesting this may explain why they did not observe a correlation of perigenual ACC with unpleasantness independent of left DLPFC activity.

A recent meta-analysis (Apkarian et al., 2005) indicates that studies investigating either clinical pain or experimental models that attempt to replicate clinical models for example capsaicin induced allodynia (Baron et al., 1999, Lorenz et al., 2002 Maihofner and Handwerker, 2005, and Zambreanu et al., 2005), have a greater likelihood of reporting PFC signal change. However approximately 55% of experimental pain studies in healthy subjects also report PFC activation (Apkarian et al., 2005). Hence, PFC activation is not uniquely associated with clinical pain. Rather, PFC activation is likely to depend on the degree to which a certain type of pain engages higher order cognitive, emotional and attentional processes (Schweinhardt et al., 2006).

Medial prefrontal areas and the perigenual cingulate are both activated by expectancy of pain (Ploghaus et al 1999, Sawamoto et al., 2000), interaction of pain with anxiety (Petrovic et al 2002) and cognitively demanding tasks (Bantick et al., 2002). Simpson et al (2001) however demonstrated a decrease in rCBF in two regions of the MPFC (Brodmann Areas 10/32 and 24/25) during anticipation related anxiety. Interestingly the changes were inversely correlated with anxiety scores so that subjects who were less anxious exhibited large rCBF reductions, whilst those with high anxiety levels showed no or minimal reduction.

Patients with bilateral lesions of the OFC, (Hornak et al 2003) demonstrate impairment in all those aspects of emotion relative to patients with lesions in the dorsolateral prefrontal cortex that did not have problems with these measures of emotion suggesting the OFC is important for aspects of emotion. The study included three patients with bilateral OFC lesions and very little medial prefrontal cortex damage that displayed deficits of voice expression identification and subjective emotional state providing evidence that damage to the OFC is sufficient to produce these changes if the lesions are bilateral.

The greater signal change observed during the muscle pain compared to cutaneous pain in the study reported here therefore lends support for the hypothesis that a nociceptive stimulus arising from muscle tissue may engage higher order cognitive, emotional and attentional processes more strongly than a nociceptive stimulus to cutaneous tissue does. Indeed this engagement may explain the absence of expected difference in the perceived affective qualities, as measured thorough the SF McGill, and unpleasantness.

Anterior Cingulate Cortex (ACC)

The ACC receives nociceptive inputs via medial thalamic nuclei (Vogt and Pandya, 1987, Vogt et al., 1987) and has extensive connections with the prefrontal cortex and parietal cortex as well as the motor system (Bush et al., 2000). Hence it has been suggested it may have a central role in processing top-down and bottom-up stimuli and assigning appropriate control to other areas in the brain.

It has been shown to code affective components of painful stimuli (Craig et al., 1996; Rainville et al., 1997; Sawamoto et al., 2000) in addition to being involved in attentional processing (Gitelman et al., 1999; Peyron et al., 1999). Furthermore it has been shown to have a role in learning associations between aversive and neutral stimuli (Ploghaus et al., 1999, Büchel et al., 2002).

Although the ACC is considered to code for unpleasantness rather than intensity of a painful stimulus, discrete intensity associated areas have been identified within the mid cingulate cortex (Coghill et al 1999, Derbyshire et al 1997).

Buchel et al's (2002) study of both innocuous and noxious laser stimulation suggest dorsal pMCC codes stimulus intensity whilst ventral pMCC (and perigenual ACC) was related to pain intensity. They report that aACC signal changes were not stimulus specific therefore suggest activation here is related to working memory and attentional processes.

Activation of both these areas, observed in the present study during both muscle and cutaneous pain conditions, with no difference in extent is therefore unsurprising given that intensity of pain was in the main, matched for the two conditions.

The perigenual anterior cingulate cortex (pACC) contains a high level of opioid receptors (Vogt et al., 1995); is located in the affective subdivision of the ACC (Bush et al., 2000) and has been linked to arousal associated with emotional/motivational processing (Critchley et al., 2004). Kalisch et al. (2005) suggest activation in medial prefrontal / pACC and anterolateral prefrontal cortex modulates anticipatory anxiety evoked by pain.

Studies of clinical pain states including angina (Rosen et al., 1994) and migraine (Weiller et al., 1995) have also reported activity in perigenual cingulate; Rosen et al. (1994) suggest the inescapable, frightening or worrying aspect of angina pain may explain the activation of perigenual cingulate.

Activation of the ventral anterior cingulate cortex, including the subgenual / perigenual areas has also been demonstrated in response to emotionally salient words (Elliott et al., 2000). Drevets (2000) suggests that the subgenual cingulate cortex has a role in the regulation of mood states, and that hypofunction of this region may confer vulnerability to mood disorders in some patients. Indeed significant changes in the subjective emotional state of patients with surgical lesions involving the pACC has also been reported (Hornak et al. (2003).

The finding in the present study of perigenual ACC signal increase during muscle pain combined with the medial PFC signal change provides support for the hypothesis that muscle pain engenders a greater affective salience than cutaneous

pain resulting in greater activation in brain regions associated with fear and anxiety.

There is however some inconsistency in the literature with regards to the pACC; increased signal has been reported during cold pain (Kwan et al., 2000) and during anticipation of a noxious stimulus (Porro et al., 2002) whilst a decrease in signal has been reported following subcutaneous ascorbic acid injections (Porro et al., 1998), during rectal distension (Dunckley et al., 2005) and hypertonic saline induced muscle pain (Henderson et al., 2006, 2007). The subgenual cingulate cortex, located more ventral to the perigenual cingulate (Vogt et al., 2003) has many interconnections with the caudal posterior cingulate cortex (Van Hoesen 1993) and signal decrease in subgenual has been reported during anticipation (Porro et al., 2002) and anxiety (Simpson et al., 2001).

A possible explanation for the reported inconsistencies may lie in semantics; within the literature there appears to be some degree of inter-changeability between perigenual and subgenual cingulate cortices when reported co-ordinates of activation are compared. Although the work of Vogt et al., (2003) clearly differentiates the two, this differentiation is not always consistently applied within the literature.

The finding of pACC activation in the present study contradicts that of Henderson and colleagues (2006 and 2007) who report a *decrease* in signal during muscle pain with no discernible change in signal during cutaneous pain. Again given that both studies employed HS as the method of pain induction, this result is somewhat surprising.

Methodological differences however between the two studies may point to reasons why the findings are different. In Henderson's study the subjects had a single injection into each tissue, separated by some 20 minutes whereas in the study reported here subjects were aware that they would receive a total of six injections and were unaware of the timing of these. It may be argued therefore that here, there was a greater degree of uncertainty, particularly during muscle pain with regards to duration and intensity of pain.

A possible explanation for the increase observed in this study therefore may well lie with expectancy. Subjects received no visual clues as to when pain was about to be induced therefore expectancy was not controlled for; this lack of control for expectancy may of course be criticised and possibly seen as a weakness in the methodology. The decision was made specifically not to manipulate expectancy with the expectation that this would be similar in both conditions.

There exists however the possibility that expectancy / anticipation in terms of degree of intensity and hence unpleasantness may have been greater during the muscle pain condition; the faster onset of cutaneous pain means that it peaked more quickly and therefore would also start to subside more quickly. The relatively slower onset of the muscle pain, it may be argued, could lead to an increased uncertainty and this may explain the difference seen in the results reported in chapter four. However, when subjects were specifically asked to attend to and rate the unpleasantness of the pain, thereby negating any expectancy effect, a sub significant increase ($Z = 2.1$) in perigenual signal was also observed during muscle but not cutaneous pain; this also showed a positive linear relationship with unpleasantness but not intensity ratings.

Whilst it is possible to control for, and / or manipulate expectancy, as others have demonstrated (Porro et al., 2002), such control relies on the ability to both predict and match the onset of a noxious stimulus. With the hypertonic saline model employed in the fMRI study reported here it was not possible to exactly match the onset curves of the muscle and cutaneous conditions and is acknowledged as a limitation of the study.

Posterior Cingulate cortex

Maddock et al (1999), in a meta-analysis of functional imaging studies demonstrated that the caudal part of the posterior cingulate cortex was consistently activated by emotional stimuli compared to matched, emotionally neutral stimuli. A confound of these studies however, is the finding that the PCC

also has a function related to episodic memory (Andreasen et al., 1995; Henson et al., 1999; Maddock et al., 2001). The authors of the meta-analysis suggest that the contrast in observations may be reconciled by the hypothesis that the posterior cingulate cortex has a role in the modulation of memory by emotionally arousing stimuli. This group later demonstrated PCC activation during both pleasant and unpleasant words; activation which was not present during recall of neutral words (Maddock et al 2003).

A recent literature review suggests PCC activation is frequently reported as being activated during pain studies of heat and mechanical stimulation of the skin, following hypnosis and suggestion and painful distension of the rectum. (Nielsen et al., 1999). Activation has also been demonstrated during distraction from pain (Valet et al 2004).

The exact role of the PCC in relation to pain processing however remains unclear. The findings of studies showing increased activation during both positive and negative affect suggest that PCC rather than reflecting the emotional valence of a stimulus it may reflect its emotional salience.

Although bilateral PCC increased signal was observed during both muscle and cutaneous pain, the activation showed a positive linear relationship with unpleasantness rating during only the muscle and not the cutaneous pain condition.

Lack of co-variance during cutaneous pain militates against a pure distraction effect; therefore it is possible the PCC signal change reflects a stronger emotional response to the muscle compared to cutaneous pain. That is the muscle condition is more salient to the individual than cutaneous pain; the later being of equal intensity but of less interest / relevance to the individual. A further possible explanation, in view of the evidence presented above regarding episodic memory is the possibility that the experimental pain evoked a memory of a previous pain experience that was emotionally salient to the individual.

Amygdala

The amygdala monitors external and internal stimuli and mediates behaviours that facilitate survival (Tillfors 2004). It is also now considered an important part of the pain system (Neugebauer et al 2004), receiving pain related information from thalamus, anterior and posterior insula, medial prefrontal cortex, anterior cingulate cortex, perigenual ACC orbitofrontal cortex and prefrontal cortex. (Millan 1999; LeDoux 2000; Price 2000; Maren and Quirck 2004; Vogt 2003).

The amygdala is heavily influenced by orbital and medial PFC; both exerting a predominantly inhibitory influence. Viewing of emotionally arousing pictures is associated with increases in activation of ventral PFC and decreased activation of the amygdala (Hari et al., 2003). Conversely, direct thalamo-amygdalar connections provide a pathway whereby rapid activation of the amygdala follows sensory stimulation, both noxious and threatening (Berntson et al., 2003)

The amygdala is also considered to be involved when orientating to motivationally salient stimuli, integrating prior experience and decision making (Rolls 2000, Baxter and Murray 2002), participating in the enhancement of both perception of, and memory for, emotionally arousing stimuli (Adolphs et al., 1997; Anderson and Phelps, 2001; Cahill et al., 1995). Rats with amygdala lesions fail to respond to either contextual or explicit cues indicative of danger such as an impending painful shock (Selden et al., 1991; Kim et al., 1993).

Animal studies suggest amygdala involvement in processing both negative and positive stimuli; however human studies provide greater evidence for amygdala involvement in negative or aversive stimuli as opposed to positive stimuli (Maddock et al., 2003).

Pain related signal changes in the amygdala have been demonstrated in a number of imaging studies in healthy human volunteers during experimental pain. However signal changes have been reported by some as showing an increase during heat pain (Bingel et al., 2002) and vascular pain (Schneider et al., 2001)

whilst others have reported a decrease in signal during heat pain (Derbyshire et al., 1997; Becerra et al., 1999).

Clinical studies present a similar story; whilst increased amygdala activation was seen in patients with irritable bowel syndrome (Naliboff et al., 2003), in neuropathic pain patients, during mechanical allodynia stimulation, bilateral amygdala de-activation was reported (Petrovic et al., 2004).

Conversely many experimental pain studies have failed to report any signal change in the amygdala. However it is worth remembering that both the fMRI BOLD signal and the rCBF changes measured in PET studies are indirect measures of brain activity i.e. they measure only the net change of associated neuronal activity to a stimulus and cannot make inferences regarding differentiation of inhibition from excitation. There has therefore been a reluctance to report a reduction in signal as 'de-activation' which may account for the lack of amygdala activation seen in much of the pain literature.

Determining the exact role of the amygdala in relation to pain processing therefore presents a challenge. Aversive conditioning studies suggest that the amygdala is only activated during the initial stages of acquisition (Quirk et al, 1997, Büchel et al, 2002) or extinction (LaBar et al., 1998) – where there is a degree of uncertainty about whether a stimulus will be aversive or neutral.

Bornhövd et al. (2002), in a study investigating response to five levels of laser stimuli ranging from non-perceived to mild warmth and strong heat pain report interesting patterns of amygdala activation. The response pattern in both the amygdala and the perigenual ACC was different from that in SII and insula cortices in that sub-threshold stimuli evoked BOLD responses almost as high as those evoked during the moderate pain stimuli. In contrast warmth and mild pain evoked minimal changes in amygdala signal.

Petrovic et al (2004) suggests that the deactivations may represent a coping strategy in dealing with acute aversive situations; reflecting a 'meaningful

suppression' of the brain systems that subserve both short-term memory and emotional response to aversive, in this case painful stimuli.

The finding in the present study of a reduction in BOLD signal in amygdala which co-varied in a linear fashion with unpleasantness ratings observed with attention to unpleasantness during the muscle but not cutaneous pain condition is of particular interest. This provides further support for the hypothesis that muscle pain may initially be more unpleasant than cutaneous pain but that the coping mechanism in healthy volunteers is such that the relative unpleasantness is relatively quickly reduced.

The decrease in amygdala signal observed may represent an inhibitory effect following increased activation in medial prefrontal cortex, MPFC, which is also more prominent during the muscle pain. MPFC in turn has been associated with monitoring one's own emotional state and anticipation of salient emotions (Ochsner et al., 2004; Erk et al., 2006), also in association with pain (Poro et al., 2002; Wiech et al., 2005)

Basal Ganglia

The basal ganglia appear to be an area of the brain where clear differences exist between muscle and cutaneous pain. During muscle pain, bilateral putamen activation was observed which was only contralateral during cutaneous pain.

Contralateral caudate activation was also observed during muscle pain with no significant change in signal during cutaneous pain.

Although bilateral putamen during attention to unpleasantness was observed during both conditions, changes in signal showed a positive correlation with unpleasantness scores in ipsilateral putamen and caudate only in the muscle pain condition.

In early brain imaging studies investigating pain, basal ganglia activation observed was frequently either not reported or attributed to either a suppression of motor response or a 'planned' motor response to the painful stimulus. However, increasingly, investigators have shown interest in the role of the basal ganglia in pain and at least one component of the basal ganglia complex feature in more

recent pain studies or indeed have been the primary focus of study (Scott et al., 2006, Wood et al., 2007).

A number of lines of evidence point to potential roles of the basal ganglia in pain processing. Putamen has been shown to encode for stimulus laterality during pain (Bingel et al 2002) and there is evidence of somatotopy in the contralateral putamen to a painful laser stimulus (Bingel et al 2004); suggesting the putamen processes behaviourally relevant nociceptive information without input from non – nociceptive receptors.

High concentrations of opiate receptors have been demonstrated in various basal ganglia nuclei in animal studies (Pasternak et al., 1975; Simantov et al., 1976; Atweh and Kuhar 1977). Furthermore in humans high levels of opiate receptor binding within the caudate nucleus and putamen have also been demonstrated (Jones et al., 1991b).

Lee and Wang (1991) report loss of pain withdrawal responses in patients who had sustained a post-traumatic basal ganglia haemorrhage in the putamen or caudate nucleus, whilst striatum infarction resulted in patients failing to respond to any sensory stimulation (Healton et al. 1982; House and Hodges 1988). Furthermore surgical lesions in the putamen alleviated phantom limb pain (Yarnitsky et al., 1988) and reduced sensation to pin-prick, light-touch and temperature (Yang, 1991).

This concomitant sensory neglect and inattention that can follow basal ganglia damage may confound studies investigating the effects of striatal or nigral lesions on pain behaviour (Chudler and Dong (1993). However Ljungberg and Ungerstedt (1976) found in rats with chemically induced substantia nigra lesions that the ability to orient to visual, olfactory and auditory stimuli are regained at different rates compared to that for somatosensory stimuli. This would suggest that the observed sensory neglect or inattention is not therefore due to a motor phenomenon. In other words, the lack of response to a stimulus may not simply be attributed to an inability, through lack of motor control, to orient to a stimulus. .

Lineberry and Vierck (1975) demonstrated in monkeys that electrical stimulation of the caudate nucleus reduces pain reactivity without any accompanying changes in escape latencies i.e. without alterations in arousal or motor ability suggesting caudate nucleus stimulation may reduce pain reactivity directly rather than reducing the ability to react. The authors attribute their results to a reduction in affective components of pain by caudate stimulation

A single case study also reports alleviation of chronic facial pain in a human by electrical stimulation of the caudate nucleus (Ervin et al. 1966), providing further evidence for a role in pain attenuation for the basal ganglia.

Motor aspects of the basal ganglia

Peyron et al (2007) in a study designed to investigate the motor component of pain reports on the central representation of the RIII reflex – a nociceptive withdrawal reflex. High intensity electrical stimulation was associated with a high-amplitude RIII reflex and bilateral brain activation in cerebellar vermis, MI, SI, and paracentral cortices and contralateral activation in premotor, SII and posterior cingulate cortices. No activation in any of the basal ganglia structures was reported.

Withdrawal assessed by the RIII nociceptive reflex, is known to be generated at the spinal-cord level and does not imply a voluntary or a conscious process (Peyron et al., 2007) therefore is an involuntary or passive response. Whilst in Peyron and colleagues' study subjects were explicitly instructed not to try and suppress any motor response, this is in contrast to most imaging studies of pain where the importance of avoiding movement during the scanning period is typically reinforced to subjects prior to entering the scanner. Basal ganglia activation observed during pain studies may still therefore represent a suppression of motor response.

Mink (1996), based on anatomical, physiological and lesion studies in both animal and humans hypothesised that 'tonically active inhibitory output of the basal ganglia acts as a "brake" on motor pattern generators (MPGs) in the cerebral

cortex (via thalamus) and brainstem'. When a movement is initiated by a particular MPG, basal ganglia output neurons projecting to competing MPGs increase their firing rate, thereby increasing inhibition and applying a "brake" on those generators. At the same time basal ganglia output neurons projecting to the areas involved with the desired movement will decrease their discharge which removes inhibition and in doing so releases the "brake" from the desired movement patterns. Selected movements are therefore enabled whilst competing movements are inhibited therefore do not interfere with that selected.

If the bilateral activation of the basal ganglia observed during muscle but not cutaneous pain represents a motor response the question is why should this be so? Pain arising in muscle tissue, as discussed previously, may from an evolutionary perspective produce a different behavioural response – that of a 'STOP' sign. If pain arising from deep tissue results in a stronger inhibitory signal, the bilateral basal ganglia activation may indicate this. However the counter argument is that cutaneous pain produces a 'flight' response i.e. removal of the painful part from danger. This response of course may be overridden by the controlled environment in which the stimulus is given.

Assessment of threat by the basal ganglia

Research in primates indicate that the basal ganglia are involved in threat assessment (Baxter, 2003; MacLean, 1972, 1990); such responses are thought to be relatively automatic, but take past experience into account, and consider appropriate behavioural responses for example 'fight or flight'.

Maclean (1990) therefore suggested that the basal ganglia represent an early threat assessment system, which still operates in primates and presumably humans. In monkeys, neuronal discharge in both caudate and anterior putamen has been shown in anticipation of predictable events (Apicella et al., 1992).

The basal ganglia are considered to function at a preconscious and pre-emotional level (Flannelly et al 2007) and assessment of threat by the basal ganglia and the

limbic system said to operate on the principle of "better to be safe than sorry" (Gilbert 2001); or put another way a false-positive with regards to whether a situation (or painful stimulus) is harmful or unsafe is better than a false-negative i.e. that a painful stimulation predicted not to be a threat actually is. The unfamiliarity of muscle pain and its association with internal, inescapable damage may reflect this.

The basal ganglia however are also a critical emotion / motor interface that allow one to translate emotional information into behavioural responses (Ring and Serra-Mestres 2002 and Grillner et al 2005). Loewenstein et al. (2001) reviewed cognitive and emotional models of information processing about potential risks or threats and concluded that "responses to risky situations (including decision making) result in part from direct (i.e., not cortically mediated) emotional influences, including feelings such as worry, fear, dread, or anxiety".

Increased threat-related basal ganglia activity may be understood as representing a state of motor readiness in response to danger. Basal ganglia activation has been reported in functional imaging studies of normal (Phelps et al., 2001) and pathological (Lorerbaum et al., 2004) fear in humans.

Butler et al (2007) also report increased activation in bilateral putamen and right caudate as well as bilateral insula and thalamus during an experimentally-induced state of conscious fear in humans which they suggest indicates involvement of both executive and motor circuits. It is tempting therefore to draw comparison's with Butler's results – the basal ganglia pattern of activation reported by this group during fear matches that observed during the muscle pain but not cutaneous pain reported here.

Limitations with regards to the motor interpretation of the results however do apply. Whilst all subjects were observed during the whole scanning period and no overt movement was observed during any pain condition, methods for assessing motor changes peripherally, for example electromyography, were not undertaken. It should also be acknowledged that whilst limiting movement (through subject instruction), improves the imaging signal to noise, it may also introduce a

potential confound. Subjects were instructed on the importance of not moving and as such, in the main, complied; this very instruction however may introduce a conscious over-riding of a desire to move in response to induced pain. It may be difficult to resolve this within a scanning environment however a well designed motor paradigm may be able to address the problem. Indeed the approach taken by Peyron et al (2007), whereby the subjects were explicitly told not to suppress any movement of their limb may be one solution.

Martin et al., (2008) recently demonstrated facilitation of motoneurones at a spinal level with the opposite effect on motor cortex outputs by group III and IV muscle afferents activated by hypertonic saline. Arguably this would allow / facilitate rest for a potentially injured muscle from which the nociceptive stimulus is arising. This would suggest an excitatory effect at the spinal level but an inhibitory effect at cortical level and perhaps questions whether the basal ganglia activation observed in the results reported here was in fact an inhibitory activation. Although it is not possible to determine whether the increase in the fMRI BOLD response is excitatory or inhibitory there is some merit in this consideration.

A body of evidence also exists for a role of forebrain dopaminergic systems in pain and analgesia (Millan 2002). Electrophysiological studies in animals have revealed dopamine-mediated inhibition of nociceptive activity in the thalamus (Shyu et al., 1992) and the spinal cord dorsal horn. Fleetwood-Walker et al., 1988; Garraway 2001). In a PET study, Jaaskelainen et al., (2001) report a significant decrease in binding of fluorodopa in the right putamen of patients with burning-mouth syndrome, suggesting decreased dopaminergic inhibition may be a feature of this chronic pain syndrome.

In a recent PET study Scott et al., (2006) investigated basal ganglia dopamine (DA) neurotransmission during a prolonged pain induced stress response, reporting significant activation of DA release in dorsal and ventral regions of the basal ganglia. Furthermore they demonstrated positive association of activation of caudate nucleus and putamen DA neurotransmission with sensory and affective ratings of induced pain; whilst nucleus accumbens DA activation was associated

with increased negative affect and fear ratings. The results suggest that basal ganglia dopamine D2 receptor-mediated neurotransmission is involved in responses to pain and may therefore contribute to variations between individuals with regards to the pain experienced.

Of note is that the experimental model of pain induction chosen in that particular study was infusion of hypertonic saline into the masseter muscle. It is possible that should the study be repeated utilising the intradermal model described in this thesis the results would be different. Such an approach may address some of the unanswered regarding the interpretation of the differences observed between cutaneous and muscle pain. Scott et al (2006) attribute the basal ganglia activation observed in the study reported above to a prolonged pain induced stress response however it is possible that this was in fact a specific response to prolonged muscle pain and the same may not be seen in cutaneous pain.

In summary it is clear that the basal ganglia have a role in pain perception, modulation and response. Whether the observed differences in basal ganglia activation described here between muscle and cutaneous pain it related to perceptual, modulatory or motor responses however remains unclear.

Cerebellum

The classical view that the cerebellum is only concerned with movement has been challenged and emerging evidence strongly suggests a role in pain. There however remains the challenge of separating the motor aspects from sensory and / or emotional response.

The output of the cerebellum originates primarily from the cerebellar nuclei (Saab and Willis 2003). Cerebellar regions involved in motor coordination receive information from the vestibular system, from the spinal cord, and from the sensorimotor cortex. Whilst other cerebellar regions receive information from frontal, parietal and occipital association areas and project back to them via the thalamus (Thach 1998). Projections from the ventral dentate nucleus of the cerebellum to the dorsal prefrontal cortex in primates has been demonstrated (Middleton and Strick 2001) providing evidence for the potential role of the cerebellum in cognitive function.

Animal studies indicate a role in pain processing; microinjection of morphine into the cerebellum in rats produces profound analgesia; reversible with administration of naloxone (Dey and Ray 1982). Whilst in monkeys, elevation of nociceptive thresholds occurs following direct stimulation of the cerebellum (Siegel and Wepsic, 1974)

Brain imaging studies have reported activation in the vermis and ipsilateral cerebellar cortex during heat pain (Casey et al., 1996, Becerra et al., 1999 and Bingel et al., 2002). Functional interconnection of the vermis with hypothalamus, amygdala, and hippocampus suggests a more complex role of the cerebellum as part of an integrated network regulating emotional behaviour (Sacchetti et al 2005).

In addition to coordinating movement, the cerebellum participates in motor learning, emotional behaviour, and fear memory. The neural correlate of cerebellar involvement in fear learning and therefore memory consolidation is provided by a behaviourally induced long-term increase of synaptic efficacy between parallel fibers and a Purkinje cell (Sacchetti et al, 2005).

Jueptner (2001) proposes, from movement studies that the cerebellum with regards to motor control is specifically concerned with feedback and monitoring of any actual movement taking place compared to the basal ganglia which have a greater role in actual or planned movement.

One possible explanation for the observation of cerebellum activation during muscle but not cutaneous pain in the present study may lie with the referred pain phenomenon associated with muscle pain. Macefield et al., (2007) recently reported cerebellum activation during HS induced muscle pain that was associated with presence of referred pain; reduced or no activation was observed in the absence of referred pain.

In the fMRI experiment reported here no data were collected regarding pain referral therefore it is not possible to determine whether the cerebellar activation observed during muscle pain was associated with referred pain. Despite results for the psychophysical experiment described in chapter three indicating that referred

pain only occurred in approximately 40% of muscle injections, it cannot be assumed to be the same during scanning.

Nevertheless, irrespective of referred pain, cerebellum activation observed during a noxious stimulus and its particular role in pain processing remains a challenge. Macefield and colleagues (2007) findings of cerebellum activation associated with referred pain is certainly interesting. The question remains as whether the cerebellum has a specific role in referred pain or whether the activation observed is related to motor intent.

6.4 Neural correlates of experimental pain in patients with lateral epicondylitis

Chapter five describes two fMRI experiments; the first involved three patients with a clinical diagnosis of lateral epicondylitis; patients were exposed, in their unaffected arm, to cutaneous and muscle noxious stimuli in the same manner as the healthy volunteers described in chapter four. The second experiment involved two of the three patients from the first experiment; these two patients were exposed to both innocuous and noxious pressure stimuli on both the affected and unaffected arms.

The author will discuss here the apparent differences observed in brain activation patterns; between healthy volunteers and patients; during presentation of an innocuous stimulus to the affected compared to the unaffected arm; and when pressure stimuli of similar intensity is presented to the affected compared to the unaffected arm. The discussion will consider how these apparent differences may be attributed to the presence in patients of an ongoing pain condition, with the caveat that small numbers allows for limited applicability and that a direct statistical comparison was not taken between health volunteer and patient data..

Methodological issues

As acknowledged previously, the main limitation with regards to the two patient experiments presented is in respect to the limited number of subjects tested.

Aside from this point; issues regarding the first experiment have already been discussed earlier in this chapter in reference to the healthy volunteer results.

An additional potential confounding factor in both of the patient experiments is the presence of background pain. All three subjects however reported their clinical pain to be predominantly a movement evoked condition; their pain being relatively quiescent at rest.

Summary of findings in patient group after hypertonic saline injection

Areas of the pain matrix were activated including ACC, MI, SI, anterior and posterior insular cortices and cerebellum during both cutaneous and muscle pain, demonstrating similarity to the healthy volunteer population.

The findings of preponderance towards bilateral activation of so called 'pain matrix' areas during muscle pain compared to an ipsilateral or contralateral tendency in cutaneous pain in the healthy volunteers also appears to be mirrored in the patient sample. Basal ganglia activation was also observed in two of the three patients during muscle but not cutaneous pain. Again this appears to reflect the findings of the healthy volunteer group. Apparent differences appear to lie with SII and the hippocampus / amygdala complex.

The extent and peak of activation of SII during the muscle pain condition in patients appeared to extend more posterior to the posterior parietal cortex in relation to the co-ordinates of peak activation in the healthy volunteer group.

Activation in the hippocampus and amygdala in all three patients during both muscle and cutaneous pain was present when compared to rest on an individual analysis basis. This may suggest greater engagement of the hippocampal complex in patients as this finding was not observed in the healthy volunteer group analysis during either cutaneous or muscle pain. Further investigation of a larger number of patients would allow a direct comparison to be undertaken and therefore further exploration of this interesting finding.

SII

SII is consistently activated in pain studies (Peyron 2000); its role suggested to relate to attention, learning and integration of nociceptive and non-nociceptive stimuli (Ploner et al., 1999).

A study by Ferretti et al., (2003) indicates that SII may be characterised by at least two neuronal populations diversely sensitive to pain; the authors demonstrating increasing activation in posterior but not anterior SII that showed a positive relationship with increasing intensity of pain. Based on their own findings and those from a previously published fMRI study investigating muscle pain induced by electrical stimulation (Niddam et al., 2002), the authors suggest parallel processing of distinct pain features in SII with respect to SI.

It is possible therefore that the observation of SII activation, apparently more posterior and more extensive in the patients, is indicative of a somatotopic shift within the parietal operculum. Cortical reorganisation of the motor and sensory homunculi in patients with Complex Regional Pain Syndrome (CRPS) (Maihofner et al 2004), phantom limb pain (Lotze et al. 1999) and chronic low back pain (Flor et al 1997) has been demonstrated. These changes have been shown not only to correlate with severity and chronicity of pain (Flor et al., 2001) but also to be reversible when pain and function improve (Maihofner et al., 2004) particularly after therapeutic interventions addressing sensory - motor incongruence (Flor 2002); the mechanism of this effect however is as yet unclear. Research in this area has tended to focus on chronic conditions, refractory to medical intervention. The finding here however in the three patients, none of whom had particularly long standing symptoms (4- 7 months), of a possible somatotopic shift would suggest further investigation in a larger clinical population is warranted.

Hippocampus

The hippocampal formation does not have a single role (Schmajuk, 1984); it would appear to integrate a number of functions that may enable it to play a central role in the organisation of behaviour (Bast and Feldon, 2003), having been shown to be involved in episodic, declarative, spatial, and contextual learning and memory. Hippocampal mechanisms have also been related to the modulation of sensorimotor processes (Gray and McNaughton, 1983, Bland and Oddie, 2001 and Vinogradova, 2001). It has been described as a 'particularly vulnerable and sensitive region' of the brain; expressing high levels of receptors for glucocorticoids or "stress" hormones (De Kloet et al., 1998). It is perhaps therefore unsurprising that the hippocampal complex has been reported as being the main area of the brain to exhibit change after repeated exposure to stressful stimuli which elevate glucocorticoid levels. (De Kloet et al., 1998; McEwen, 2005).

The hippocampus has also been associated with the protective and defensive reactions to threatening and aversive stimuli, and hyperfunction of some hippocampal processes has been proposed as contributing to anxiety disorders.

It has been hypothesised that anxiety may be linked to a decrease in $GABA_A$ receptor-mediated inhibition in the hippocampus (Low et al., 2000); some support for this hypothesis comes from a rat study in which a chemically induced reduction in local $GABA_A$ receptor-mediated inhibition through hippocampal MK-801 infusion resulted in a significant increase in the animals' startle reactivity (Zhang et al., 2005).

Bast and Feldon (2003) suggest that although the hippocampus processes sensory input in order to create memory, through contributions to spatial memory it may directly modulate the translation of sensory input into motor responses; the integration of mnemonic and sensorimotor functions perhaps serving to turn memory or experience into action. Indeed the anatomical substrate exists for integration of sensory motor function; highly processed input is received in projections from the sensory association cortices predominantly to the dorsal hippocampus (Moser and Moser, 1998).

Early animal studies also revealed a role for the hippocampus in nociception; lesions in rats increase the vocalisation threshold to painful stimuli (Blanchard and Fial, 1968) whilst electrical stimulation of the hippocampus increases tail-flick latency (Prado, 1985) and lidocaine injections into the dentate gyrus produce analgesia (Yeung et al., 1977, McKenna and Melzack, 1992). Hyperfunction of some hippocampal processes, possibly due to reduced inhibitory GABA transmission within the hippocampus, has also been suggested to contribute significantly to clinical symptoms of anxiety disorders (Löw et al., 2000).

The hippocampus and amygdala in addition to prefrontal cortex all show morphological changes as a result of stress-related disorders such as depression and PTSD. Animal models of repeated psychosocial and restraint stress suggests there are multiple mechanisms for changes in volume of brain structures; these include neuronal damage, glial cell loss, dendritic remodelling and reduced dentate gyrus granule cell number (McEwen 2005). Hornyak et al., (2007) report slightly increased gray matter density in the ventral hippocampus in patients with restless leg syndrome.

Kuchinad et al., (2007), in turn report reduced gray matter density in fibromyalgia patients compared to healthy controls in a number of brain regions including cingulate, insular and medial frontal cortices and parahippocampal gyri; the reduction correlating with duration of symptoms.

The hippocampal complex therefore has been implicated in both stress and nociception. Activation during experimental pain studies however is rarely reported. The observation therefore of increased signal in the hippocampal complex in all three patients presented here during both cutaneous and muscle pain is of interest. Whether this represents an increase in stress experienced by the patients in relation to that experienced by the healthy volunteers is a question that cannot be answered from the results here but perhaps sets a challenge for future research.

That an increase in amygdala activation was observed in the patients as opposed to the decrease see in the healthy volunteer group is also particularly interesting. If, as suggested by Petrovic et al (2004), that deactivation (as seen in the healthy volunteers) represent a coping strategy in dealing with acute aversive situations the question must be asked whether the three patients has poorer coping strategies?
Conversely it could be argued that the presence of an ongoing pain condition negates the need for acute coping strategies in the presence of a pain that is less emotionally salient in comparison to an ongoing pain condition. Further studies to investigate this area would be wise to gather a wider psychometric profile of subjects, whether they be patients or healthy volunteers.

Brain activation patterns observed in subject C are of particular interest. Despite reporting low pain scores (mean peak of 3.9 and 3.1 for ID and MS respectively) activation was still observed in amongst others pACC, insula, SI, PFC and hippocampus. The findings lend further weight to the hypothesis that the presence of an ongoing pain syndrome enhances brain activation processing of a new noxious stimulus; even when that stimulus induces subjectively relatively mild levels of pain.

Typically, clinical imaging studies have utilised a noxious stimulus very distinct from the ongoing pain condition, for example heat, as the acute pain stimulus. Hypertonic saline was chosen here as previous reports suggest the properties of the resulting pain reflect those of clinical musculoskeletal pain (Arendt Nielsen et al., 1996). Lateral epicondylitis was also specifically chosen as the clinical condition of interest for this study as it was felt to represent a clinical condition of musculoskeletal pain that is focal and well defined; it also allowed for induction of pain in similar tissues on the contralateral, unaffected arm.

It is tempting to hypothesise that by selecting the mirrored site for pain induction, in addition to the HS model producing a more tonic pain, that brain activation patterns observed in the patients; in particular the hippocampal activation reflects an increase stress response or perhaps even evoked a memory of their own pain.

Again both the low numbers and study design of course do not allow that interpretation to be made; however future studies may wish to consider this.

Summary of findings associated with noxious and innocuous pressure

During noxious pressure on both the affected and unaffected arms in two patients with tennis elbow, widespread brain activation was observed in areas consistent with previous reports of pressure pain. When stimulus intensity but not pain intensity was matched, greater activation was observed during stimulus application to the affected arm than the unaffected arm. Areas of activation included; SI, SII, MI, ACC, anterior and posterior insular cortices, hippocampus and cerebellum.

The observation of greater activation on pressure application to the affected arm during the intensity matched contrast is consistent with the findings of previous studies employing a similar technique in fibromyalgia patients (Gracely et al., 2002) and low back pain patients (Giesecke et al., 2004).

The findings provide further evidence that brain activation patterns observed during pain studies reflect the intensity of the pain experienced by the individual rather than the intensity of the stimulus.

The final observations relate to the widespread activation seen during application of innocuous pressure to the affected arm; areas which again included the prefrontal cortex and amygdala / hippocampal complex are of particular interest.

Medial prefrontal areas and the perigenual cingulate are both activated by expectancy of pain (Ploghaus et al 1999, Sawamoto et al., 2000), whilst aversive conditioning studies report amygdala activation during phases where there is a degree of uncertainty about whether an impending stimulus will be aversive or neutral (Quirk et al, 1997, Büchel et al, 1998b, 1999).

The paradigm was such that subjects were aware that they would be receiving various stimuli throughout the scanning period; some of which would be painful and some non-painful. Therefore a degree of expectancy / anticipation and or anxiety could be expected, but should in theory be no different between sides.

The results however suggest an increased fear / anxiety response during anticipation of an actual or potential pain is greater when the stimulus is anatomically located in close proximity to their 'own' pain.

Both patients were interviewed immediately after the end of scanning to assess whether a summation effect occurred during the pressure pain paradigm in either the affected or unaffected side; both denied any significant increase in background pain.

6.6 What this thesis has contributed to the body of knowledge

The work described within this thesis contributes to the body of knowledge in three key areas:

i. Experimental pain models – psychophysical properties and influence of tissue type.

Despite frequent references in the literature to perceptual differences in pain arising from muscle and cutaneous tissue, direct comparisons of pain arising from these two tissue types have been limited and flawed.

The results of the experiment investigating psychophysical properties of deep and superficial pain (chapter three) have demonstrated that intradermal hypertonic saline is a reliable and valid method of acute pain induction. Although common in animal studies, there are no previous reports in the literature of a systematic investigation utilising hypertonic saline intradermally (as opposed to subcutaneously) in humans.

It supports previous reports in the literature of perceptual differences between these two tissue types but adds further weight by removing the confounding effect of different types of stimuli.

Conversely it has also highlighted the unpredictability of a subcutaneous model; future studies maybe wise to target intradermal rather than subcutaneous tissue

Despite published accounts in the literature (Svensson et al., 1997a, Rainville et al., 1992), of muscle pain being perceived as more unpleasant than cutaneous pain, the results here question these previous reports. A possible explanation for the finding here may be that the tool used to investigate unpleasantness may not be sensitive enough to reveal subtle differences. Alternatively in a controlled environment, when the subject is aware that the pain induced is of limited duration, any difference in relative unpleasantness between the two tissue types is in fact insignificant. It is acknowledged however that as only one measure of unpleasantness per injection was taken, differences related to the temporal pattern may not have been detected.

ii. Experimental pain models – neural correlates and influence of tissue type.

When the author embarked on this course of study, a review of the literature revealed few studies investigating brain activation patterns associated with muscle pain; even less that compared muscle and cutaneous pain.

Henderson et al., (2006) highlighted this issue and undertook a comparison of hypertonic saline in muscle and cutaneous tissue; the first to do so. Whilst acknowledging the contribution by this group it should be noted that subcutaneous hypertonic saline was utilised as the cutaneous pain model. In view of the results reported here in chapter 3, their findings should be interpreted with caution.

The study reported in chapter four therefore is the first to undertake a direct comparison of brain activation patterns in superficial and deep tissue pain using intradermal hypertonic saline as the experimental model for superficial tissue pain.

The results suggest that muscle would appear to engage more affective and cognitive processing than cutaneous pain; specifically in prefrontal regions and the basal ganglia. The finding of no difference in perceived relative unpleasantness however perhaps makes the interpretation of the fMRI results more challenging.

The findings presented reflect some but not all of the findings of Henderson and colleagues (2006) in relation to brain processing of muscle and cutaneous pain in healthy volunteers. Again, whilst acknowledging the work of Henderson's group, it may be argued that the experiments here provide a greater insight into the differences between deep and superficial pain by (i) utilising an intradermal model and (ii) implementing a repeated injection model, undertaken in one scanning run, as opposed to single muscle and cutaneous injections.

Schweinhardt et al's (2006) suggestion, from their review of the literature, that rostral anterior insula may be a key area where differences are observed between clinical and experimental pain are questionable based on the findings of chapter four. Here, rostral anterior insula activation was observed during both cutaneous and muscle pain. Although the activation appeared more rostral during muscle pain there was no difference when the co-ordinates of peak activation were compared. These results therefore have also served to highlight that caution should be observed when making comparisons between experimental and clinical pain; consideration must be given to the experimental model – both in relation to tissue involved and the temporal characteristics of the induced pain. The over reliance of researchers on brief, experimental models of pain may have over sold differences between experimental and clinical pain.

Whilst extremely unlikely that any experimental model can completely encapture the nature, behaviour, or psychophysical impact of a clinical pain condition, the author believes that the results presented in this thesis lend support for the suggestion that experimental muscle pain more closely reflects a number of the processes involved in clinical pain processing than does experimental cutaneous pain.

iii. Experimental pain models in the presence of lateral epicondylitis.

Finally, albeit in a limited number of patients, the findings of augmented processing in patients with a seemingly relatively benign pain condition to experimental pain, stimulation of clinical pain and in particular anticipation of clinical pain raises a number of interesting questions and directions for future research.

The literature review served to highlight the lack of studies that have investigated clinical pain populations. Those involving patient populations have focussed on conditions where the underlying pathophysiology is elusive or indeed where 'psychological factors' are suggested to have a greater role in both severity of symptoms and response to therapeutic intervention. Interestingly this is also a point raised in a recent review (Schweinhardt and Tracey (2007); the authors highlighting the focus of clinical pain imaging studies on chronic pain conditions which are relatively ill-defined at least in terms of pathophysiology, citing fibromyalgia, chronic low back pain and irritable bowel syndrome as examples.

The findings presented here in this small group of patients with a relatively well defined clinical pain syndrome highlight the need for further investigation.

Clinical studies have tended to either investigate experimental pain distinct and distal from the area of clinical pain, making comparisons with the same experimental pain in healthy volunteers, or attempted to replicate clinical pain. The latter can be particularly difficult in the scanning environment. The unilateral nature of lateral epicondylitis allows the patient to serve as their own control and so comparisons between brain processing of experimental and clinical pain may be made. Future investigations in a larger population of patients with lateral epicondylitis would expand on the findings reported here.

Implications for future research and clinical practice

Patrick Wall (2000) proposed that pain is not simply a sensation but a need state like hunger or thirst; as such the processing and response involves cognitive, affective, and sensory components. If indeed one considers pain as a need like state; the next question may be are the 'needs' of muscle and cutaneous pain different? The findings reported in this thesis would suggest that the brain certainly interprets and responds to noxious stimuli arising from these two tissues in a different manner.

An organism when subjected to an external stimulus is required to analyse the stimulus, determine and then execute an appropriate response. When considered from a behavioural perspective the differences observed between muscle and cutaneous pain reported in this thesis may well reflect the differences between interoceptive and exteroceptive processing, that is the consequences and response to an external versus an internal threat. Taken from a motor control perspective the difference may lie in facilitating escape from further damage / danger or conversely in inhibiting motor output and therefore reduce further damage and or promoted healing and recovery.

The work presented in this thesis has demonstrated that there are differences in both the psychophysical properties and brain activation patterns associated with muscle and cutaneous pain. Studies that investigate further the psychophysical properties including the temporal aspects of relative unpleasantness and other psychometric properties including kinesiophobia and catastrophizing would add to the discussion and interpretation of these results. Well thought out motor control studies, again utilising the two tissues would also add significantly to the debate about the key differences between cutaneous and muscle pain.

Significant impact on clinical care of patients with chronic pain is unlikely to be an immediate outcome of this research. Nevertheless, there is certainly clinical applicability; perhaps more so in questions that it asks rather than answers it provides.

Despite there being a greater acknowledgement amongst health care professionals regarding psychosocial factors impacting on the symptoms and quality of life inpatients with pain, the management, particularly in the acute stages still tends to be focussed on peripheral tissues. This is especially true when considering this from a diagnostic point of view. Clinical diagnostics relies heavily on findings from physical examination - and the patient's response to the same.

The finding therefore of widespread activation during innocuous pressure to the affected side in the two patients reported here would sound a note of caution. Furthermore, many physiotherapists regularly practice manual therapy techniques that involve the repeated application of light pressure – typically non pain provoking. Again here the question would be asked what is the brain's response to such techniques? Furthermore, despite the patient not reporting an immediate increase in symptoms, is there a risk that therapy maybe augmenting widespread brain activation and so hindering rather than helping recovery?

These are certainly questions of interest to clinicians, patients and researchers alike and the author would suggest valuable questions to pursue.

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Appendix A

Volunteer and Patient information sheets

THE UNIVERSITY
of LIVERPOOL

Pain Research Institute
Dept of Neurological Science
Clinical Sciences Centre for
Research and Education
Lower Lane, Fazakerley
Liverpool L9 7LJ

Heather Cameron
Research Fellow

Telephone: 0151 5295965

h.cameron@liv.ac.uk

22 Nov 2004

VOLUNTEER INFORMATION SHEET

Title of Study: Psychophysical properties of experimental cutaneous and muscle pain

Researchers: Ms Cameron, Dr Harmon, Dr Tripathi, Prof. Nurmikko

You are being invited to participate in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Take time to decide whether or not you wish to take part.

Consumers in Ethics in Research (CERES) publish a leaflet entitled "Medical Research and You". This leaflet gives you more information about medical research and addresses some questions you may want to ask. The leaflet may be obtained from CERES, PO BOX 1365, London N16 0BW.

Thank you for reading this information sheet.

What is the purpose of this study?

This study has been designed to evaluate the differences between superficial (skin) pain and deep (muscle) pain. Chronic pain is a common condition which is often difficult to treat effectively. By studying experimental pain in healthy volunteers we can learn more about the underlying mechanisms of pain and therefore design better treatments. You are being invited to take part in this project as a healthy volunteer.

Previous studies in healthy volunteers have typically used experimentally induced pain in the skin. Whilst these studies have helped our understanding of pain mechanisms they may not give a true picture when we compare it to patients with chronic pain.

We would like to compare skin pain with muscle pain as we believe muscle pain will tell us more about the underlying mechanisms.

How do I know if I am eligible?

We expect you to answer NO to a simple question: "Do you suffer from neck or arm pain or any other chronic or recurrent pain?"
If you have occasional headaches, or have suffered in the past an acute episode of neck or arm pain lasting less than 7 days in the past (but not in the last 6 months) you are eligible.

What will happen to me if I take part?

If you agree we will ask you to come to the Pain Research Institute, Clinical Sciences Centre, University Hospital Aintree on two occasions at weekly intervals. We will first ask you some general questions about your health and well-being. After that we will ask you to complete the following:

1. Three small needles will be inserted into your forearm - one into a muscle and two just in the skin. Before inserting the needle into your muscle you will first have a local anaesthetic cream placed on your skin for 30 minutes to numb the area.
2. You will then receive two infusions of a small amount (0.2 ml) of saline through each needle. Hypertonic saline although not harmful causes a short lasting pain when injected into muscle or skin. Each injection produces a moderate degree of pain which lasts 3 - 4 minutes.
3. Whilst the pain is present we will
    i. ask you to tell us about the pain in terms of intensity and unpleasantness and where you feel the sensation. We will also ask you to fill in a brief questionnaire to describe the quality of the pain.
    ii. test the sensitivity of your skin in the area surrounding the needle by lightly brushing across it a few times.
    iii. Assess the affect of the pain on the blood flow around your needle using a machine called a laser Doppler. This in itself is not painful.

What do I have to do next?
If you are interested in taking part you should contact Heather Cameron (details on back page) who will answer any questions you may have. If you then decide to take part an appointment will be made for you to come to the Pain Research Institute.

What are the possible disadvantages and risks of taking part?

We do not expect any major side effects. However whenever a needle is inserted into muscle or skin there is a small risk of bleeding which may cause some discomfort / bruising that can persist for a few days. There is also a very small risk that you could develop an infection where the needle was inserted. Using a small disposable needle, inserted using an aseptic technique reduces this risk.

What are the possible benefits of taking part?
There is no direct benefit to you. By carrying out this type of research we hope to find new ways of treating patients with chronic pain.

What if something goes wrong?

This study is being undertaken by staff of The University of Liverpool. In the unlikely event that your participation will result in disease, illness or bodily injury, you may be entitled to compensation, under the indemnity arrangements that have been agreed between the University of Liverpool and Royal & SunAlliance Insurance Company. The copy of these arrangements will be provided on request.
If you are during the course of this study harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the university will have your name and address removed so that you cannot be recognised from it. We will not routinely inform you GP of your participation, but will be happy to do so if you so wish.

What will happen to the results of the study?

We will test several healthy volunteers. All the results will be compiled and evaluated on a group rather than individual basis. We expect to publish these results in international scientific journals that focus on pain.

Contact for Further Information
Heather Cameron, Research Fellow, Pain Research Institute, University of Liverpool, Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL tel 0151 529 5822

Prof. T J Nurmikko, Honorary Consultant in Pain Relief, Pain Research Institute, University of Liverpool Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL, tel 0151 529 5820

Thank you for your time

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of LIVERPOOL

Pain Research Institute
Dept of Neurological Science
Clinical Sciences Centre for
Research and Education
Lower Lane, Fazakerley
Liverpool L9 7LJ

The Walton Centre NHS
for Neurology and Neurosurgery
NHS Trust

Heather Cameron
Research Fellow
Specialist Physiotherapist
Telephone: 0151 5295835

h.cameron@liv.ac.uk

25 May 2005

VOLUNTEER INFORMATION SHEET

Title of Study: Functional MRI of the brain in chronic tennis elbow pain

Researchers: Ms Cameron, Prof. Roberts, Dr Zaman, Prof. Nurmikko

You are being invited to participate in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Take time to decide whether or not you wish to take part.

Consumers in Ethics in Research (CERES) publish a leaflet entitled "Medical Research and You". This leaflet gives you more information about medical research and addresses some questions you may want to ask. The leaflet may be obtained from CERES, PO BOX 1365, London N16 0BW.

Thank you for reading this information sheet.

What is the purpose of this study?

This study has been designed to evaluate how the human brain handles painful information. We plan to carry out brain imaging in patients with chronic elbow pain. We use functional MRI, which is capable of detecting the shape and size of active brain structures. You are being invited to this project as a healthy volunteer.

Previous scans of healthy volunteers have found that some areas of the brain light up when a painful stimulus is applied however this brain activity changes a little depending upon the type of painful stimulus. We would like to compare these phenomena in patients with clinical pain and in healthy volunteers with experimentally induced pain.

How do I know if I am eligible?
We expect you to answer NO to a simple question: "Do you suffer from neck or arm pain or any other chronic or recurrent pain?" If you have occasional headaches, or have suffered in the past an acute episode of neck or arm pain lasting less than 7 days in the past (but not in the last 6 months) you are eligible.

What will happen to me if I take part?

If you agree we will ask you to come to the Magnetic Resonance Imaging and Analysis Centre (MARIARC). We will first ask you some general questions about your health and well-being. After that we will ask you to complete a set of tasks. These will be similar to the ones you will be subjected to later, while you are being scanned. The tasks include the following:

1. First having four plastic cannulae inserted via a needle into your forearm – two into a muscle and two into your skin. You will then receive an infusion of hypertonic saline.
Hypertonic saline although not harmful can cause a short lasting pain when injected into a muscle or skin. The aim is to produce moderate rather than intense pain. The pain is expected to last less than two minutes but will be repeated up to two times in each cannula. During the scan we will ask you to rate your pain on an electronic scale.

2. Lifting your hand against a resistance. You will be asked to do this task with both hands but one at a time. The aim again is to rekindle a moderate pain in the arm we previously injected lasting only 15 seconds. It is unlikely that this task will produce any pain in the arm that was not injected.

3. Having your hands moved passively by one of the investigators. This may cause mild discomfort.

You will be wearing earplugs while in the scanner to protect your hearing - the MRI scanner makes quite a bit of noise!

These tasks will be repeated while you are in the scanner. Each stimulus will be given to you at set intervals.

What do I have to do?
We will inform you when to come for the scan. You will first have to be checked by one of our nurses that you have no contra-indications to having the scan.

What are the possible disadvantages and risks of taking part?

We do not expect any major side effects. However whenever a needle is inserted into a muscle or skin there is a small risk of bleeding which may cause some discomfort / bruising that can persist for a few days. There is also a very small risk that you could develop an infection where the needle was inserted. Using a small disposable needle, inserted using an aseptic technique reduces this risk.
We are not aware of any real risks associated with scanning. There is a very remote possibility that the scan will reveal an abnormality of the brain, which has not given any symptoms. In such an unlikely event, we feel it is our obligation to inform you. If you cannot agree to this before the scan, we will not enter you into the study. (As already mentioned, your treatment will remain the same irrespective of whether or not you participate in this study).

We ask you to wear earplugs in order to protect you ears from the noise of the scanner. Whilst there is no real risk of hearing loss, you may experience temporary impairment of hearing after the scan.

What are the possible benefits of taking part?
There is no direct benefit to you. By carrying out this type of research we hope to find new ways of treating patients with chronic pain.

What if something goes wrong?
This study is a joint project of The University of Liverpool and The Walton Centre for Neurology and Neurosurgery NHS Trust. In the unlikely event that your participation will result in disease, illness or bodily injury, you may be entitled to compensation, under the indemnity arrangements that have been agreed between the University of Liverpool and Royal & SunAlliance Insurance Company. The copy of these arrangements will be provided on request.

The NHS does not have similar compensation arrangements. However, if you are during the course of this study harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this research, the National Health Service complaints mechanism may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. We will not routinely inform you GP of your participation, but will be happy to do so if you so wish.

What will happen to the results of the study?

We will scan several people with elbow pain, as well as some healthy volunteers. All the results will be compiled and evaluated on a group rather than individual basis. We expect to publish these results in international scientific journals that focus on pain and brain imaging.

Contact for Further Information
Heather Cameron, Research Fellow, Pain Research Institute, Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL. Tel 0151 529 5822

Prof. T J Nurmikko, Honorary Consultant in Pain Relief, Pain Research Institute, Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL, tel 0151 529 5820

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Dept of Neurological Science
Clinical Sciences Centre for
Research and Education
Lower Lane, Fazakerley
Liverpool L9 7LJ

Heather Cameron
Research Fellow
Specialist Physiotherapist
Telephone: 0151 5295835

h.cameron@liv.ac.uk

28 Oct 2006

PATIENT INFORMATION SHEET

Title of Study: Functional MRI of the brain in chronic tennis elbow pain

Researchers: Ms Cameron, Prof. Roberts, Dr. Zaman, Prof. Nurmikko.

You are being invited to participate in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your friends, relatives and your GP if you wish. Take time to decide whether or not you wish to take part.

Consumers in Ethics in Research (CERES) publish a leaflet entitled "Medical Research and You". This leaflet gives you more information about medical research and addresses some questions you may want to ask. The leaflet may be obtained from CERES, PO BOX 1365, London N16 0BW.

Thank you for reading this information sheet.

What is the purpose of this study?

This study has been designed to evaluate how the human brain handles painful information. We plan to carry out brain imaging in patients with chronic tennis elbow pain. We use functional MRI, which is capable of detecting the shape and size of active brain structures. We hope to perform this scan in two groups of people: healthy volunteers and those like yourself suffering from pain due to tennis elbow.

Why have I been chosen?

You have chronic elbow pain which is one of our clinical and research interests. We wish to explore the reactions of the brain in this condition in the hope that it would yield new insight into this very common problem.

Do I have to take part?

It is up to you whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree we will ask you to come to the Magnetic Resonance Imaging and Analysis Centre (MARIARC) on two separate days approximately one week apart. We will first ask you some general questions about your health and well-being. After that we will ask you to complete a set of tasks. These will be similar to the ones you will be subjected to later, while you are being scanned. On the first visit you will:

Have four plastic cannulae inserted via a needle into your forearm (the arm which does not have tennis elbow) – two into a muscle and two into your skin. You will then receive an injection of hypertonic saline. Hypertonic saline although not harmful can cause a short lasting pain when injected into a muscle or skin. The aim is to produce moderate rather than intense pain. The pain is expected to last less than two minutes but will be repeated up to two times in each cannula whilst you are scanned. During the scan we will ask you to rate your pain on an electronic scale.

On you second visit we will repeat the procedure carried out on your first visit and also ask you to carry out the following task during scanning:

1. Lifting your hand against a resistance. You will be asked to do this task with both hands but one at a time. The aim again is to rekindle a moderate pain in the arm we previously injected lasting only 30 seconds. It is unlikely that this task will produce any pain in the arm that was not injected.

2. Having your hands moved passively by one of the investigators. This may cause mild discomfort.

3. Receiving a painful pressure stimulus applied to your forearm muscle of both your left and right arm. Each stimulus will last 15 seconds, and will be repeated 10 times. The aim is to produce moderate rather than intense pain.

You will be wearing earplugs while in the scanner to protect your hearing - the MRI scanner makes quite a bit of noise!

These tasks will be repeated while you are in the scanner. Each stimulus will be given to you at set intervals. Prior to each set you will be told which stimulus to expect.

What do I have to do now?

You will have to do nothing. We will inform you when to come to the Pain Research Institute. After you have completed the first set of tasks we will take you to the Scanner Room and carry out the brain scan.

What are the possible disadvantages and risks of taking part?

There is a potential risk, whenever a needle is inserted into a muscle or skin of bleeding which may lead to some discomfort / bruising that can persist for a few days. There is also a very small risk that you could develop an infection where the needle was inserted. Using a small disposable needle, inserted using an aseptic technique reduces this risk.
We are not aware of any real risks associated with scanning. There is a very remote possibility that the scan will reveal an abnormality of the brain, which has not given any symptoms. In such an unlikely event, we

feel it is our obligation to inform you. If you cannot agree to this before the scan, we will not enter you into the study. (As already mentioned, your treatment will remain the same irrespective of whether or not you participate in this study).

We ask you to wear earplugs in order to protect you ears from the noise of the scanner. Whilst there is no real risk of hearing loss, you may experience temporary impairment of hearing after the scan.

What are the possible benefits of taking part?

There is no direct benefit to you. By carrying out this type of research we hope to find new ways of treating patients with chronic tennis elbow pain and similar conditions.

What if something goes wrong?

This study is a joint project of The University of Liverpool and The Walton Centre for Neurology and Neurosurgery NHS Trust. In the unlikely event that your participation will result in disease, illness or bodily injury, you may be entitled to compensation, under the indemnity arrangements that have been agreed between the University of Liverpool and Royal & SunAlliance Insurance Company. The copy of these arrangements will be provided at request.

The NHS does not have similar compensation arrangements. However, if you are during the course of this study harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this research, the National Health Service complaints mechanism may be available to you.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. Unless you disagree, your GP will be informed of your participation and will receive information about the nature and purpose of the study.

What will happen to the results of the study?

We will scan several people with similar types of elbow pain, as well as some healthy volunteers. All the results will be compiled and evaluated on a group basis rather than individually. We expect to publish these results in international scientific journals that focus on pain and brain imaging.

Contact for Further Information

Heather Cameron, Research Fellow, Pain Research Institute, Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL, tel 0151 529 5822

Prof. T J Nurmikko, Honorary Consultant in Pain Relief, Pain Research Institute, Clinical Sciences Centre, Lower Lane, Liverpool L9 7AL,

Appendix B

SPSS output from chapter 3

General Linear Model

Within-Subjects Factors

Measure: intensity

injection	time	Dependent Variable
1	1	int_1
	2	int_2
	3	int_3
	4	int_4
2	1	sub_1
	2	sub_2
	3	sub_3
	4	sub_4
3	1	mus_1
	2	mus_2
	3	mus_3
	4	mus_4

Descriptive Statistics

	Mean	Std. Deviation	N
int_1	5.5938	2.09936	16
int_2	5.0563	2.37458	16
int_3	5.6375	1.83589	16
int_4	5.7000	1.78885	16
sub_1	4.6563	2.27302	16
sub_2	2.3875	1.74198	16
sub_3	5.2938	1.80683	16
sub_4	4.1688	2.46271	16
mus_1	5.2500	1.83303	16
mus_2	4.5563	1.87402	16
mus_3	5.5625	2.15310	16
mus_4	4.5938	2.04335	16

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
injection	Pillai's Trace	.687	15.391[a]	2.000	14.000	.000	.687
	Wilks' Lambda	.313	15.391[a]	2.000	14.000	.000	.687
	Hotelling's Trace	2.199	15.391[a]	2.000	14.000	.000	.687
	Roy's Largest Root	2.199	15.391[a]	2.000	14.000	.000	.687
time	Pillai's Trace	.628	7.322[a]	3.000	13.000	.004	.628
	Wilks' Lambda	.372	7.322[a]	3.000	13.000	.004	.628
	Hotelling's Trace	1.690	7.322[a]	3.000	13.000	.004	.628
	Roy's Largest Root	1.690	7.322[a]	3.000	13.000	.004	.628
injection * time	Pillai's Trace	.647	3.050[a]	6.000	10.000	.058	.647
	Wilks' Lambda	.353	3.050[a]	6.000	10.000	.058	.647
	Hotelling's Trace	1.830	3.050[a]	6.000	10.000	.058	.647
	Roy's Largest Root	1.830	3.050[a]	6.000	10.000	.058	.647

a. Exact statistic

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Mauchly's Test of Sphericity[b]

Measure: intensity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
injection	.929	1.026	2	.599	.934	1.000	.500
time	.592	7.203	5	.207	.737	.871	.333
injection * time	.072	33.600	20	.033	.619	.849	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Tests of Within-Subjects Effects

Measure: intensity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Sphericity Assumed	61.454	2	30.727	13.201	.000	.468
	Greenhouse-Geisser	61.454	1.868	32.898	13.201	.000	.468
	Huynh-Feldt	61.454	2.000	30.727	13.201	.000	.468
	Lower-bound	61.454	1.000	61.454	13.201	.002	.468
Error(injection)	Sphericity Assumed	69.829	30	2.328			
	Greenhouse-Geisser	69.829	28.021	2.492			
	Huynh-Feldt	69.829	30.000	2.328			
	Lower-bound	69.829	15.000	4.655			
time	Sphericity Assumed	59.597	3	19.866	7.967	.000	.347
	Greenhouse-Geisser	59.597	2.212	26.938	7.967	.001	.347
	Huynh-Feldt	59.597	2.612	22.819	7.967	.000	.347
	Lower-bound	59.597	1.000	59.597	7.967	.013	.347
Error(time)	Sphericity Assumed	112.202	45	2.493			
	Greenhouse-Geisser	112.202	33.186	3.381			
	Huynh-Feldt	112.202	39.176	2.864			
	Lower-bound	112.202	15.000	7.480			
injection * time	Sphericity Assumed	31.189	6	5.198	2.415	.033	.139
	Greenhouse-Geisser	31.189	3.716	8.394	2.415	.064	.139
	Huynh-Feldt	31.189	5.091	6.126	2.415	.043	.139
	Lower-bound	31.189	1.000	31.189	2.415	.141	.139
Error(injection*time)	Sphericity Assumed	193.715	90	2.152			
	Greenhouse-Geisser	193.715	55.735	3.476			
	Huynh-Feldt	193.715	76.367	2.537			
	Lower-bound	193.715	15.000	12.914			

Tests of Within-Subjects Contrasts

Measure: intensity

Source	injection	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Linear		8.201	1	8.201	3.617	.077	.194
	Quadratic		53.253	1	53.253	22.301	.000	.598
Error(injection)	Linear		34.011	15	2.267			
	Quadratic		35.818	15	2.388			
time		Linear	.509	1	.509	.138	.716	.009
		Quadratic	2.876	1	2.876	2.285	.151	.132
		Cubic	56.212	1	56.212	22.207	.000	.597
Error(time)		Linear	55.349	15	3.690			
		Quadratic	18.885	15	1.259			
		Cubic	37.969	15	2.531			
injection * time	Linear	Linear	1.388	1	1.388	.314	.583	.021
		Quadratic	1.531	1	1.531	2.269	.153	.131
		Cubic	1.661	1	1.661	1.136	.303	.070
	Quadratic	Linear	1.160	1	1.160	.444	.516	.029
		Quadratic	2.568	1	2.568	1.325	.268	.081
		Cubic	22.881	1	22.881	12.642	.003	.457
Error(injection*time)	Linear	Linear	66.205	15	4.414			
		Quadratic	10.121	15	.675			
		Cubic	21.932	15	1.462			
	Quadratic	Linear	39.241	15	2.616			
		Quadratic	29.067	15	1.938			
		Cubic	27.149	15	1.810			

Tests of Between-Subjects Effects

Measure: intensity

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	4556.178	1	4556.178	183.862	.000	.925
Error	371.707	15	24.780			

Estimated Marginal Means

1. injection

Estimates

Measure: intensity

injection	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	5.497	.383	4.681	6.312
2	4.127	.411	3.251	5.002
3	4.991	.381	4.179	5.802

Pairwise Comparisons

Measure: intensity

(I) injection	(J) injection	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	1.370*	.239	.000	.727	2.014
	3	.506	.266	.230	-.211	1.223
2	1	-1.370*	.239	.000	-2.014	-.727
	3	-.864*	.301	.035	-1.674	-.054
3	1	-.506	.266	.230	-1.223	.211
	2	.864*	.301	.035	.054	1.674

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.687	15.391[a]	2.000	14.000	.000	.687
Wilks' lambda	.313	15.391[a]	2.000	14.000	.000	.687
Hotelling's trace	2.199	15.391[a]	2.000	14.000	.000	.687
Roy's largest root	2.199	15.391[a]	2.000	14.000	.000	.687

Each F tests the multivariate effect of injection. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

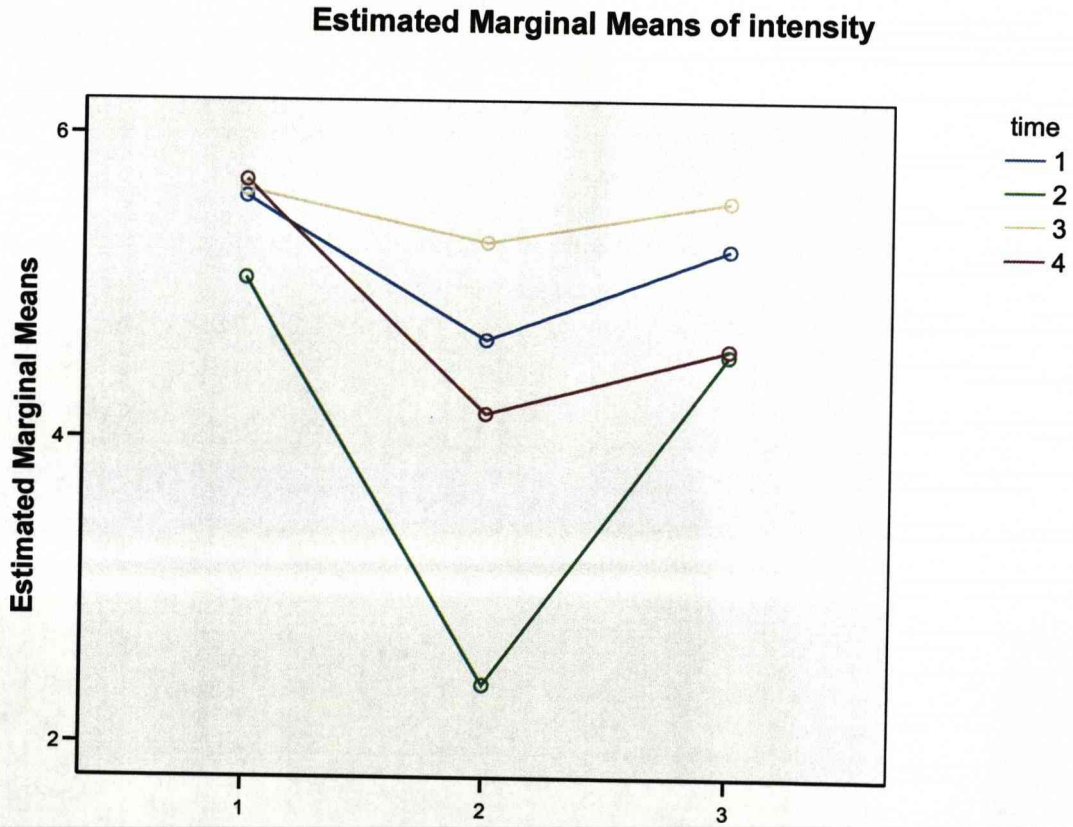
a. Exact statistic

3. injection * time

Measure: intensity

injection	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	5.594	.525	4.475	6.712
	2	5.056	.594	3.791	6.322
	3	5.638	.459	4.659	6.616
	4	5.700	.447	4.747	6.653
2	1	4.656	.568	3.445	5.867
	2	2.388	.435	1.459	3.316
	3	5.294	.452	4.331	6.257
	4	4.169	.616	2.856	5.481
3	1	5.250	.458	4.273	6.227
	2	4.556	.469	3.558	5.555
	3	5.563	.538	4.415	6.710
	4	4.594	.511	3.505	5.683

Profile Plots



General Linear Model

Within-Subjects Factors

Measure: unpleasantness

injection	time	Dependent Variable
1	1	unp_int_1
	2	unp_int_2
	3	unp_int_3
	4	unp_int_4
2	1	unp_sub_1
	2	unp_sub_2
	3	unp_sub_3
	4	unp_sub_4
3	1	unp_mus_1
	2	unp_mus_2
	3	unp_mus_3
	4	unpl_mus_4

Descriptive Statistics

	Mean	Std. Deviation	N
unp_int_1	4.7500	2.54296	16
unp_int_2	4.0000	2.75681	16
unp_int_3	5.2500	2.01660	16
unp_int_4	4.6875	1.95683	16
unp_sub_1	3.6875	2.08866	16
unp_sub_2	1.8125	2.07264	16
unp_sub_3	5.0625	2.32289	16
unp_sub_4	3.4375	1.93111	16
unp_mus_1	4.6250	2.21736	16
unp_mus_2	3.1875	2.19754	16
unp_mus_3	5.3750	1.99583	16
unpl_mus_4	3.1875	2.04022	16

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
injection	Pillai's Trace	.507	7.212[a]	2.000	14.000	.007	.507
	Wilks' Lambda	.493	7.212[a]	2.000	14.000	.007	.507
	Hotelling's Trace	1.030	7.212[a]	2.000	14.000	.007	.507
	Roy's Largest Root	1.030	7.212[a]	2.000	14.000	.007	.507
time	Pillai's Trace	.722	11.245[a]	3.000	13.000	.001	.722
	Wilks' Lambda	.278	11.245[a]	3.000	13.000	.001	.722
	Hotelling's Trace	2.595	11.245[a]	3.000	13.000	.001	.722
	Roy's Largest Root	2.595	11.245[a]	3.000	13.000	.001	.722
injection * time	Pillai's Trace	.438	1.298[a]	6.000	10.000	.341	.438
	Wilks' Lambda	.562	1.298[a]	6.000	10.000	.341	.438
	Hotelling's Trace	.779	1.298[a]	6.000	10.000	.341	.438
	Roy's Largest Root	.779	1.298[a]	6.000	10.000	.341	.438

a. Exact statistic

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Mauchly's Test of Sphericity[b]

Measure: unpleasantness

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
injection	.925	1.091	2	.579	.930	1.000	.500
time	.645	6.011	5	.306	.774	.924	.333
injection * time	.186	21.521	20	.382	.612	.835	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Tests of Within-Subjects Effects

Measure: unpleasantness

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Sphericity Assumed	43.948	2	21.974	5.648	.008	.274
	Greenhouse-Geisser	43.948	1.860	23.622	5.648	.010	.274
	Huynh-Feldt	43.948	2.000	21.974	5.648	.008	.274
	Lower-bound	43.948	1.000	43.948	5.648	.031	.274
Error(injection)	Sphericity Assumed	116.719	30	3.891			
	Greenhouse-Geisser	116.719	27.907	4.182			
	Huynh-Feldt	116.719	30.000	3.891			
	Lower-bound	116.719	15.000	7.781			
time	Sphericity Assumed	127.557	3	42.519	12.276	.000	.450
	Greenhouse-Geisser	127.557	2.322	54.923	12.276	.000	.450
	Huynh-Feldt	127.557	2.773	45.994	12.276	.000	.450
	Lower-bound	127.557	1.000	127.557	12.276	.003	.450
Error(time)	Sphericity Assumed	155.859	45	3.464			
	Greenhouse-Geisser	155.859	34.837	4.474			
	Huynh-Feldt	155.859	41.601	3.747			
	Lower-bound	155.859	15.000	10.391			
injection * time	Sphericity Assumed	27.427	6	4.571	1.759	.117	.105
	Greenhouse-Geisser	27.427	3.672	7.469	1.759	.155	.105
	Huynh-Feldt	27.427	5.011	5.474	1.759	.132	.105
	Lower-bound	27.427	1.000	27.427	1.759	.205	.105
Error(injection*time)	Sphericity Assumed	233.906	90	2.599			
	Greenhouse-Geisser	233.906	55.085	4.246			
	Huynh-Feldt	233.906	75.158	3.112			
	Lower-bound	233.906	15.000	15.594			

Tests of Within-Subjects Contrasts

Measure: unpleasantness

Source	injection	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Linear		10.695	1	10.695	2.302	.150	.133
	Quadratic		33.253	1	33.253	10.604	.005	.414
Error(injection)	Linear		69.680	15	4.645			
	Quadratic		47.039	15	3.136			
time		Linear	.551	1	.551	.118	.736	.008
		Quadratic	.130	1	.130	.066	.800	.004
		Cubic	126.876	1	126.876	33.680	.000	.692
Error(time)		Linear	69.899	15	4.660			
		Quadratic	29.453	15	1.964			
		Cubic	56.507	15	3.767			
injection * time	Linear	Linear	4.064	1	4.064	.671	.426	.043
		Quadratic	1.758	1	1.758	1.220	.287	.075
		Cubic	7.014	1	7.014	3.658	.075	.196
	Quadratic	Linear	4.901	1	4.901	2.408	.142	.138
		Quadratic	.753	1	.753	.480	.499	.031
		Cubic	8.938	1	8.938	3.478	.082	.188
Error(injection*time)	Linear	Linear	90.911	15	6.061			
		Quadratic	21.617	15	1.441			
		Cubic	28.761	15	1.917			
	Quadratic	Linear	30.524	15	2.035			
		Quadratic	23.539	15	1.569			
		Cubic	38.554	15	2.570			

Tests of Between-Subjects Effects

Measure: unpleasantness

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	3209.505	1	3209.505	134.447	.000	.900
Error	358.078	15	23.872			

Estimated Marginal Means

1. injection

Estimates

Measure: unpleasantness

injection	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	4.672	.445	3.723	5.621
2	3.500	.359	2.734	4.266
3	4.094	.409	3.222	4.965

Pairwise Comparisons

Measure: unpleasantness

(I) injection	(J) injection	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	1.172*	.299	.004	.366	1.977
	3	.578	.381	.450	-.448	1.604
2	1	-1.172*	.299	.004	-1.977	-.366
	3	-.594	.361	.362	-1.566	.378
3	1	-.578	.381	.450	-1.604	.448
	2	.594	.361	.362	-.378	1.566

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.507	7.212[a]	2.000	14.000	.007	.507
Wilks' lambda	.493	7.212[a]	2.000	14.000	.007	.507
Hotelling's trace	1.030	7.212[a]	2.000	14.000	.007	.507
Roy's largest root	1.030	7.212[a]	2.000	14.000	.007	.507

Each F tests the multivariate effect of injection. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

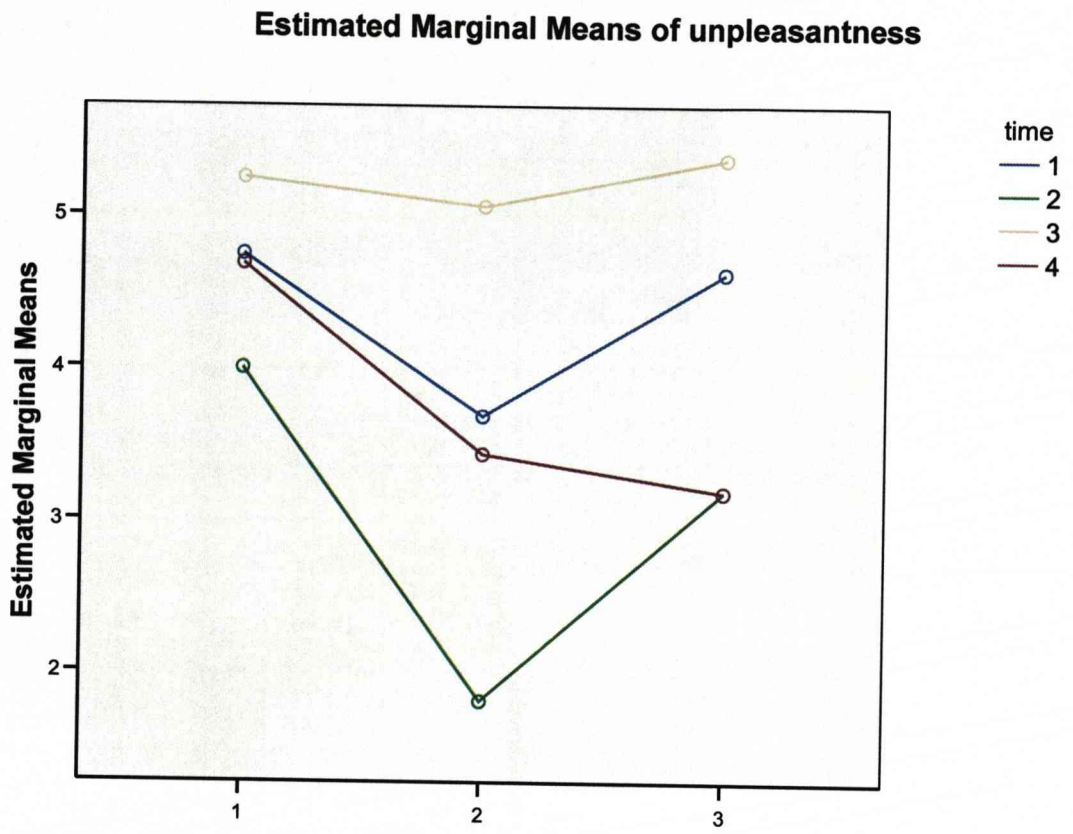
2. time

3. injection * time

Measure: unpleasantness

injection	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	4.750	.636	3.395	6.105
	2	4.000	.689	2.531	5.469
	3	5.250	.504	4.175	6.325
	4	4.688	.489	3.645	5.730
2	1	3.688	.522	2.575	4.800
	2	1.813	.518	.708	2.917
	3	5.063	.581	3.825	6.300
	4	3.438	.483	2.408	4.467
3	1	4.625	.554	3.443	5.807
	2	3.188	.549	2.017	4.358
	3	5.375	.499	4.311	6.439
	4	3.188	.510	2.100	4.275

Profile Plots



General Linear Model

Within-Subjects Factors

Measure: timetopeakvas

injection	time	Dependent Variable
1	1	T_int1
	2	T_int2
	3	T_int3
	4	T_int4
2	1	T_sub1
	2	T_sub2
	3	T_sub3
	4	T_sub4
3	1	T_mus1
	2	T_mus2
	3	T_mus3
	4	T_mus4

Descriptive Statistics

	Mean	Std. Deviation	N
T_int1	36.7500	13.34416	16
T_int2	27.9375	12.70941	16
T_int3	35.1875	15.50148	16
T_int4	27.0625	13.61112	16
T_sub1	42.8750	11.27165	16
T_sub2	36.0000	13.42138	16
T_sub3	44.5000	12.73316	16
T_sub4	37.1875	17.15505	16
T_mus1	46.8750	13.84136	16
T_mus2	47.0000	16.00833	16
T_mus3	43.6875	10.93446	16
T_mus4	42.6875	14.50388	16

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
injection	Pillai's Trace	.577	9.532[a]	2.000	14.000	.002	.577
	Wilks' Lambda	.423	9.532[a]	2.000	14.000	.002	.577
	Hotelling's Trace	1.362	9.532[a]	2.000	14.000	.002	.577
	Roy's Largest Root	1.362	9.532[a]	2.000	14.000	.002	.577
time	Pillai's Trace	.241	1.374[a]	3.000	13.000	.295	.241
	Wilks' Lambda	.759	1.374[a]	3.000	13.000	.295	.241
	Hotelling's Trace	.317	1.374[a]	3.000	13.000	.295	.241
	Roy's Largest Root	.317	1.374[a]	3.000	13.000	.295	.241
injection * time	Pillai's Trace	.324	.799[a]	6.000	10.000	.592	.324
	Wilks' Lambda	.676	.799[a]	6.000	10.000	.592	.324
	Hotelling's Trace	.479	.799[a]	6.000	10.000	.592	.324
	Roy's Largest Root	.479	.799[a]	6.000	10.000	.592	.324

a. Exact statistic

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Mauchly's Test of Sphericity[b]

Measure: timetopeakvas

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
injection	.873	1.902	2	.386	.887	.998	.500
time	.823	2.679	5	.750	.892	1.000	.333
injection * time	.215	19.612	20	.497	.713	1.000	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.

Design: Intercept
Within Subjects Design: injection+time+injection*time

Tests of Within-Subjects Effects

Measure: timetopeakvas

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Sphericity Assumed	5813.948	2	2906.974	13.851	.000	.480
	Greenhouse-Geisser	5813.948	1.775	3276.231	13.851	.000	.480
	Huynh-Feldt	5813.948	1.996	2913.305	13.851	.000	.480
	Lower-bound	5813.948	1.000	5813.948	13.851	.002	.480
Error(injection)	Sphericity Assumed	6296.052	30	209.868			
	Greenhouse-Geisser	6296.052	26.619	236.527			
	Huynh-Feldt	6296.052	29.935	210.325			
	Lower-bound	6296.052	15.000	419.737			
time	Sphericity Assumed	1434.042	3	478.014	2.071	.117	.121
	Greenhouse-Geisser	1434.042	2.676	535.812	2.071	.125	.121
	Huynh-Feldt	1434.042	3.000	478.014	2.071	.117	.121
	Lower-bound	1434.042	1.000	1434.042	2.071	.171	.121
Error(time)	Sphericity Assumed	10387.792	45	230.840			
	Greenhouse-Geisser	10387.792	40.146	258.752			
	Huynh-Feldt	10387.792	45.000	230.840			
	Lower-bound	10387.792	15.000	692.519			
injection * time	Sphericity Assumed	809.802	6	134.967	.997	.432	.062
	Greenhouse-Geisser	809.802	4.276	189.390	.997	.419	.062
	Huynh-Feldt	809.802	6.000	134.967	.997	.432	.062
	Lower-bound	809.802	1.000	809.802	.997	.334	.062
Error(injection*time)	Sphericity Assumed	12182.865	90	135.365			
	Greenhouse-Geisser	12182.865	64.138	189.948			
	Huynh-Feldt	12182.865	90.000	135.365			
	Lower-bound	12182.865	15.000	812.191			

Tests of Within-Subjects Contrasts

Measure: timetopeakvas

Source	injection	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Linear		5684.445	1	5684.445	20.177	.000	.574
	Quadratic		129.503	1	129.503	.938	.348	.059
Error(injection)	Linear		4225.930	15	281.729			
	Quadratic		2070.122	15	138.008			
time		Linear	570.417	1	570.417	2.901	.109	.162
		Quadratic	1.021	1	1.021	.005	.947	.000
		Cubic	862.604	1	862.604	3.183	.095	.175
Error(time)		Linear	2949.683	15	196.646			
		Quadratic	3373.479	15	224.899			
		Cubic	4064.629	15	270.975			
injection * time	Linear	Linear	14.102	1	14.102	.160	.695	.011
		Quadratic	6.570	1	6.570	.052	.823	.003
		Cubic	553.164	1	553.164	3.397	.085	.185
	Quadratic	Linear	56.376	1	56.376	.260	.617	.017
		Quadratic	.128	1	.128	.002	.961	.000
		Cubic	179.463	1	179.463	1.080	.315	.067
Error(injection*time)	Linear	Linear	1321.773	15	88.118			
		Quadratic	1901.805	15	126.787			
		Cubic	2442.711	15	162.847			
	Quadratic	Linear	3251.549	15	216.770			
		Quadratic	772.497	15	51.500			
		Cubic	2492.529	15	166.169			

Tests of Between-Subjects Effects

Measure: timetopeakvas

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	291720.083	1	291720.083	763.476	.000	.981
Error	5731.417	15	382.094			

Estimated Marginal Means

1. injection

Estimates

Measure: timetopeakvas

injection	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	31.734	2.019	27.431	36.038
2	40.141	2.023	35.828	44.453
3	45.063	2.087	40.613	49.512

Pairwise Comparisons

Measure: timetopeakvas

(I) injection	(J) injection	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-8.406*	2.449	.011	-15.003	-1.809
	3	-13.328*	2.967	.001	-21.321	-5.335
2	1	8.406*	2.449	.011	1.809	15.003
	3	-4.922	2.208	.124	-10.869	1.025
3	1	13.328*	2.967	.001	5.335	21.321
	2	4.922	2.208	.124	-1.025	10.869

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.577	9.532[a]	2.000	14.000	.002	.577
Wilks' lambda	.423	9.532[a]	2.000	14.000	.002	.577
Hotelling's trace	1.362	9.532[a]	2.000	14.000	.002	.577
Roy's largest root	1.362	9.532[a]	2.000	14.000	.002	.577

Each F tests the multivariate effect of injection. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

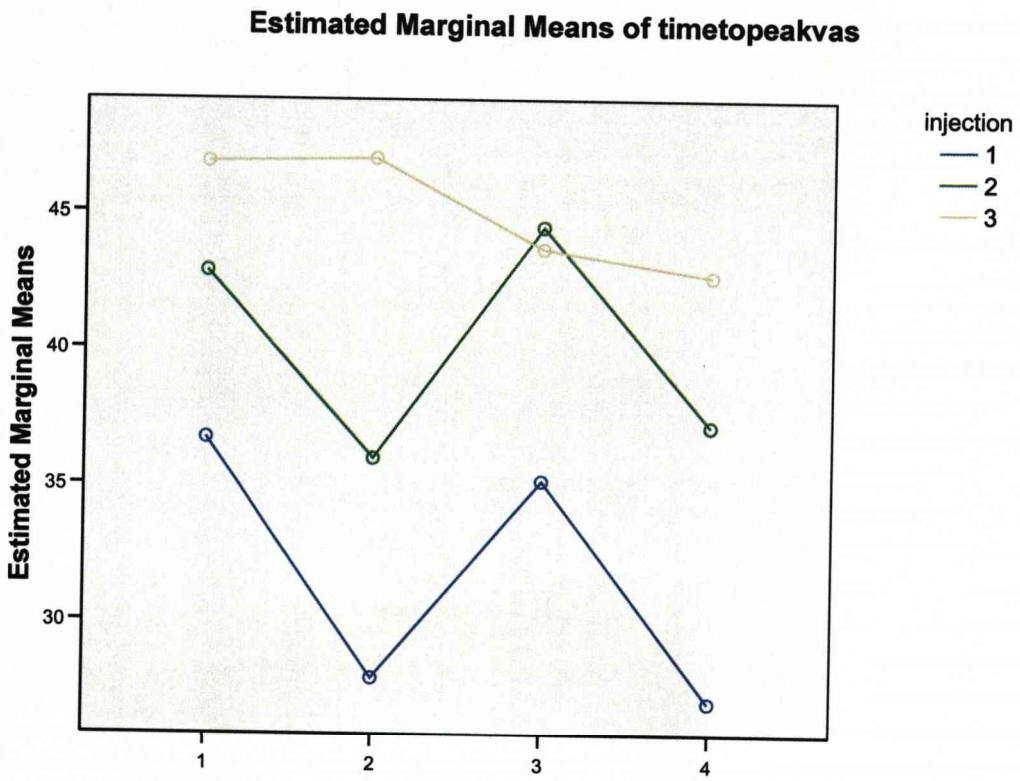
2. time

3. injection * time

Measure: timetopeakvas

injection	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	36.750	3.336	29.639	43.861
	2	27.938	3.177	21.165	34.710
	3	35.188	3.875	26.927	43.448
	4	27.063	3.403	19.810	34.315
2	1	42.875	2.818	36.869	48.881
	2	36.000	3.355	28.848	43.152
	3	44.500	3.183	37.715	51.285
	4	37.188	4.289	28.046	46.329
3	1	46.875	3.460	39.499	54.251
	2	47.000	4.002	38.470	55.530
	3	43.688	2.734	37.861	49.514
	4	42.688	3.626	34.959	50.416

Profile Plots



General Linear Model

Within-Subjects Factors

Measure: A60

injection	time	Dependent Variable
1	1	A60_int1
	2	A60_int2
	3	A60_int3
	4	A60_int4
2	1	A60_sub1
	2	A60_sub2
	3	A60_sub3
	4	A60_sub4
3	1	A60_mus1
	2	A60_mus2
	3	A60_mus3
	4	A60_mus4

Descriptive Statistics

	Mean	Std. Deviation	N
A60_int1	281.7533	119.22889	15
A60_int2	247.7467	144.38024	15
A60_int3	271.4800	116.08545	15
A60_int4	288.6867	118.85930	15
A60_sub1	198.1267	111.67750	15
A60_sub2	92.2667	95.11626	15
A60_sub3	230.0867	101.52957	15
A60_sub4	192.9400	136.50066	15
A60_mus1	188.3533	103.57024	15
A60_mus2	161.0733	106.77591	15
A60_mus3	220.7067	127.65094	15
A60_mus4	156.1667	112.69460	15

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
injection	Pillai's Trace	.815	28.697[a]	2.000	13.000	.000	.815
	Wilks' Lambda	.185	28.697[a]	2.000	13.000	.000	.815
	Hotelling's Trace	4.415	28.697[a]	2.000	13.000	.000	.815
	Roy's Largest Root	4.415	28.697[a]	2.000	13.000	.000	.815
time	Pillai's Trace	.593	5.834[a]	3.000	12.000	.011	.593
	Wilks' Lambda	.407	5.834[a]	3.000	12.000	.011	.593
	Hotelling's Trace	1.459	5.834[a]	3.000	12.000	.011	.593
	Roy's Largest Root	1.459	5.834[a]	3.000	12.000	.011	.593
injection * time	Pillai's Trace	.465	1.305[a]	6.000	9.000	.345	.465
	Wilks' Lambda	.535	1.305[a]	6.000	9.000	.345	.465
	Hotelling's Trace	.870	1.305[a]	6.000	9.000	.345	.465
	Roy's Largest Root	.870	1.305[a]	6.000	9.000	.345	.465

a. Exact statistic

b.

Design: Intercept
Within Subjects Design: injection+time+injection*time

Mauchly's Test of Sphericity[b]

Measure: A60

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
injection	.932	.921	2	.631	.936	1.000	.500
time	.667	5.143	5	.400	.775	.938	.333
injection * time	.189	19.601	20	.501	.628	.887	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.

Design: Intercept
Within Subjects Design: injection+time+injection*time

Tests of Within-Subjects Effects

Measure: A60

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Sphericity Assumed	342203.479	2	171101.739	23.099	.000	.623
	Greenhouse-Geisser	342203.479	1.872	182801.178	23.099	.000	.623
	Huynh-Feldt	342203.479	2.000	171101.739	23.099	.000	.623
	Lower-bound	342203.479	1.000	342203.479	23.099	.000	.623
Error(injection)	Sphericity Assumed	207409.160	28	7407.470			
	Greenhouse-Geisser	207409.160	26.208	7913.971			
	Huynh-Feldt	207409.160	28.000	7407.470			
	Lower-bound	207409.160	14.000	14814.940			
time	Sphericity Assumed	133167.560	3	44389.187	5.887	.002	.296
	Greenhouse-Geisser	133167.560	2.325	57288.091	5.887	.005	.296
	Huynh-Feldt	133167.560	2.815	47304.399	5.887	.002	.296
	Lower-bound	133167.560	1.000	133167.560	5.887	.029	.296
Error(time)	Sphericity Assumed	316665.892	42	7539.664			
	Greenhouse-Geisser	316665.892	32.543	9730.590			
	Huynh-Feldt	316665.892	39.412	8034.824			
	Lower-bound	316665.892	14.000	22618.992			
injection * time	Sphericity Assumed	81265.163	6	13544.194	1.579	.163	.101
	Greenhouse-Geisser	81265.163	3.765	21583.729	1.579	.196	.101
	Huynh-Feldt	81265.163	5.323	15267.813	1.579	.173	.101
	Lower-bound	81265.163	1.000	81265.163	1.579	.230	.101
Error(injection*time)	Sphericity Assumed	720720.285	84	8580.003			
	Greenhouse-Geisser	720720.285	52.712	13672.905			
	Huynh-Feldt	720720.285	74.517	9671.885			
	Lower-bound	720720.285	14.000	51480.020			

Tests of Within-Subjects Contrasts

Measure: A60

Source	injection	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Linear		247566.252	1	247566.252	41.177	.000	.746
	Quadratic		94637.227	1	94637.227	10.751	.005	.434
Error(injection)	Linear		84171.502	14	6012.250			
	Quadratic		123237.658	14	8802.690			
time		Linear	4216.338	1	4216.338	.358	.559	.025
		Quadratic	8542.222	1	8542.222	1.915	.188	.120
		Cubic	120409.000	1	120409.000	18.841	.001	.574
Error(time)		Linear	164748.521	14	11767.752			
		Quadratic	62448.113	14	4460.579			
		Cubic	89469.258	14	6390.661			
injection * time	Linear	Linear	2488.399	1	2488.399	.117	.737	.008
		Quadratic	14676.620	1	14676.620	3.129	.099	.183
		Cubic	8083.542	1	8083.542	1.489	.243	.096
	Quadratic	Linear	7015.991	1	7015.991	1.072	.318	.071
		Quadratic	9528.540	1	9528.540	1.318	.270	.086
		Cubic	39472.070	1	39472.070	6.236	.026	.308
Error(injection*time)	Linear	Linear	297597.100	14	21256.936			
		Quadratic	65664.499	14	4690.321			
		Cubic	76003.011	14	5428.786			
	Quadratic	Linear	91586.820	14	6541.916			
		Quadratic	101246.956	14	7231.925			
		Cubic	88621.899	14	6330.136			

Tests of Between-Subjects Effects

Measure: A60

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	7997246.137	1	7997246.137	106.159	.000	.883
Error	1054661.625	14	75332.973			

Estimated Marginal Means

1. injection

Estimates

Measure: A60

injection	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	272.417	23.930	221.091	323.742
2	178.355	21.577	132.077	224.633
3	181.575	21.546	135.363	227.787

Pairwise Comparisons

Measure: A60

(I) injection	(J) injection	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	94.062*	15.211	.000	52.721	135.402
	3	90.842*	14.157	.000	52.368	129.316
2	1	-94.062*	15.211	.000	-135.402	-52.721
	3	-3.220	17.577	1.000	-50.990	44.550
3	1	-90.842*	14.157	.000	-129.316	-52.368
	2	3.220	17.577	1.000	-44.550	50.990

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.815	28.697[a]	2.000	13.000	.000	.815
Wilks' lambda	.185	28.697[a]	2.000	13.000	.000	.815
Hotelling's trace	4.415	28.697[a]	2.000	13.000	.000	.815
Roy's largest root	4.415	28.697[a]	2.000	13.000	.000	.815

Each F tests the multivariate effect of injection. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

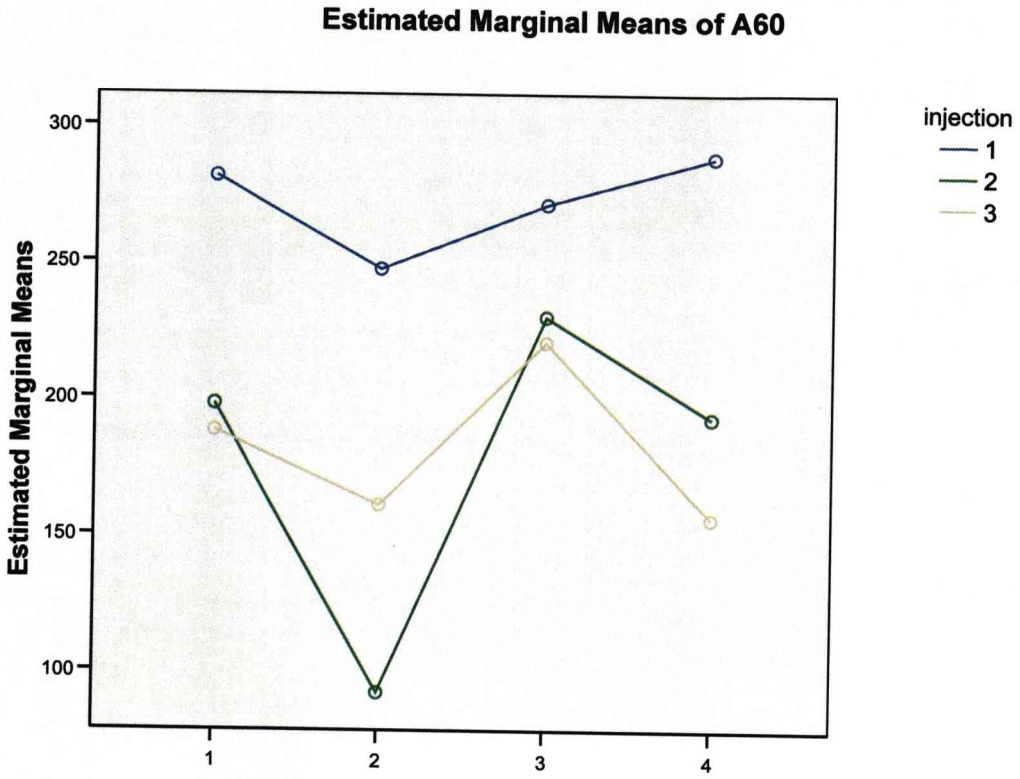
2. time

3. injection * time

Measure: A60

injection	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	281.753	30.785	215.727	347.780
	2	247.747	37.279	167.792	327.702
	3	271.480	29.973	207.194	335.766
	4	288.687	30.689	222.865	354.509
2	1	198.127	28.835	136.282	259.972
	2	92.267	24.559	39.593	144.940
	3	230.087	26.215	173.861	286.312
	4	192.940	35.244	117.348	268.532
3	1	188.353	26.742	130.998	245.709
	2	161.073	27.569	101.943	220.204
	3	220.707	32.959	150.016	291.397
	4	156.167	29.098	93.758	218.575

Profile Plots



General Linear Model

Within-Subjects Factors

Measure: AR

injection	time	Dependent Variable
1	1	AR_int1
	2	AR_int2
	3	AR_int3
	4	AR_int4
2	1	AR_sub1
	2	AR_sub2
	3	AR_sub3
	4	AR_sub4
3	1	AR_mus1
	2	AR_mus2
	3	AR_mus3
	4	AR_mus4

Descriptive Statistics

	Mean	Std. Deviation	N
AR_int1	702.1200	348.94487	15
AR_int2	566.9733	336.29282	15
AR_int3	643.2667	281.57130	15
AR_int4	633.1533	257.24708	15
AR_sub1	651.6267	337.97205	15
AR_sub2	282.3800	252.99994	15
AR_sub3	697.2200	272.53295	15
AR_sub4	513.0133	358.57342	15
AR_mus1	623.0000	340.47502	15
AR_mus2	470.9667	280.51496	15
AR_mus3	689.6467	331.49488	15
AR_mus4	435.0800	317.43825	15

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
injection	Pillai's Trace	.400	4.326[a]	2.000	13.000	.036	.400
	Wilks' Lambda	.600	4.326[a]	2.000	13.000	.036	.400
	Hotelling's Trace	.666	4.326[a]	2.000	13.000	.036	.400
	Roy's Largest Root	.666	4.326[a]	2.000	13.000	.036	.400
time	Pillai's Trace	.651	7.456[a]	3.000	12.000	.004	.651
	Wilks' Lambda	.349	7.456[a]	3.000	12.000	.004	.651
	Hotelling's Trace	1.864	7.456[a]	3.000	12.000	.004	.651
	Roy's Largest Root	1.864	7.456[a]	3.000	12.000	.004	.651
injection * time	Pillai's Trace	.646	2.739[a]	6.000	9.000	.085	.646
	Wilks' Lambda	.354	2.739[a]	6.000	9.000	.085	.646
	Hotelling's Trace	1.826	2.739[a]	6.000	9.000	.085	.646
	Roy's Largest Root	1.826	2.739[a]	6.000	9.000	.085	.646

a. Exact statistic

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Mauchly's Test of Sphericity[b]

Measure: AR

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[a]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
injection	.964	.473	2	.789	.965	1.000	.500
time	.437	10.543	5	.062	.768	.928	.333
injection * time	.099	27.260	20	.140	.506	.662	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

b.
Design: Intercept
Within Subjects Design: injection+time+injection*time

Tests of Within-Subjects Effects

Measure: AR

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Sphericity Assumed	341718.624	2	170859.312	4.266	.024	.234
	Greenhouse-Geisser	341718.624	1.931	176967.724	4.266	.026	.234
	Huynh-Feldt	341718.624	2.000	170859.312	4.266	.024	.234
	Lower-bound	341718.624	1.000	341718.624	4.266	.058	.234
Error(injection)	Sphericity Assumed	1121439.616	28	40051.415			
	Greenhouse-Geisser	1121439.616	27.034	41483.298			
	Huynh-Feldt	1121439.616	28.000	40051.415			
	Lower-bound	1121439.616	14.000	80102.830			
time	Sphericity Assumed	1704479.110	3	568159.703	11.287	.000	.446
	Greenhouse-Geisser	1704479.110	2.304	739841.004	11.287	.000	.446
	Huynh-Feldt	1704479.110	2.784	612324.136	11.287	.000	.446
	Lower-bound	1704479.110	1.000	1704479.110	11.287	.005	.446
Error(time)	Sphericity Assumed	2114267.276	42	50339.697			
	Greenhouse-Geisser	2114267.276	32.254	65550.886			
	Huynh-Feldt	2114267.276	38.971	54252.724			
	Lower-bound	2114267.276	14.000	151019.091			
injection * time	Sphericity Assumed	659602.900	6	109933.817	2.130	.058	.132
	Greenhouse-Geisser	659602.900	3.035	217349.656	2.130	.110	.132
	Huynh-Feldt	659602.900	3.969	166187.684	2.130	.090	.132
	Lower-bound	659602.900	1.000	659602.900	2.130	.166	.132
Error(injection*time)	Sphericity Assumed	4335001.907	84	51607.166			
	Greenhouse-Geisser	4335001.907	42.487	102032.296			
	Huynh-Feldt	4335001.907	55.566	78014.896			
	Lower-bound	4335001.907	14.000	309642.993			

Tests of Within-Subjects Contrasts

Measure: AR

Source	injection	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
injection	Linear		200271.211	1	200271.211	4.275	.058	.234
	Quadratic		141447.413	1	141447.413	4.253	.058	.233
Error(injection)	Linear		655843.978	14	46845.998			
	Quadratic		465595.638	14	33256.831			
time		Linear	56807.545	1	56807.545	.877	.365	.059
		Quadratic	53841.065	1	53841.065	2.780	.118	.166
		Cubic	1593830.501	1	1593830.501	23.842	.000	.630
Error(time)		Linear	907211.556	14	64800.825			
		Quadratic	271178.986	14	19369.928			
		Cubic	935876.733	14	66848.338			
injection * time	Linear	Linear	17249.554	1	17249.554	.122	.733	.009
		Quadratic	97099.852	1	97099.852	3.349	.089	.193
		Cubic	111839.915	1	111839.915	5.082	.041	.266
	Quadratic	Linear	28047.382	1	28047.382	.693	.419	.047
		Quadratic	75507.410	1	75507.410	1.374	.261	.089
		Cubic	329858.786	1	329858.786	15.550	.001	.526
Error(injection*time)	Linear	Linear	1987486.976	14	141963.355			
		Quadratic	405903.402	14	28993.100			
		Cubic	308128.728	14	22009.195			
	Quadratic	Linear	566938.859	14	40495.633			
		Quadratic	769566.273	14	54969.019			
		Cubic	296977.670	14	21212.691			

Tests of Between-Subjects Effects

Measure: AR

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	59658294.2	1	59658294.18	95.316	.000	.872
Error	8762598.375	14	625899.884			

Estimated Marginal Means

1. injection

Estimates

Measure: AR

injection	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
1	636.378	61.508	504.456	768.301
2	536.060	62.754	401.466	670.654
3	554.673	63.603	418.259	691.088

Pairwise Comparisons

Measure: AR

(I) injection	(J) injection	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	100.318*	33.545	.029	9.151	191.486
	3	81.705	39.516	.173	-25.690	189.100
2	1	-100.318*	33.545	.029	-191.486	-9.151
	3	-18.613	36.309	1.000	-117.292	80.065
3	1	-81.705	39.516	.173	-189.100	25.690
	2	18.613	36.309	1.000	-80.065	117.292

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.400	4.326[a]	2.000	13.000	.036	.400
Wilks' lambda	.600	4.326[a]	2.000	13.000	.036	.400
Hotelling's trace	.666	4.326[a]	2.000	13.000	.036	.400
Roy's largest root	.666	4.326[a]	2.000	13.000	.036	.400

Each F tests the multivariate effect of injection. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Exact statistic

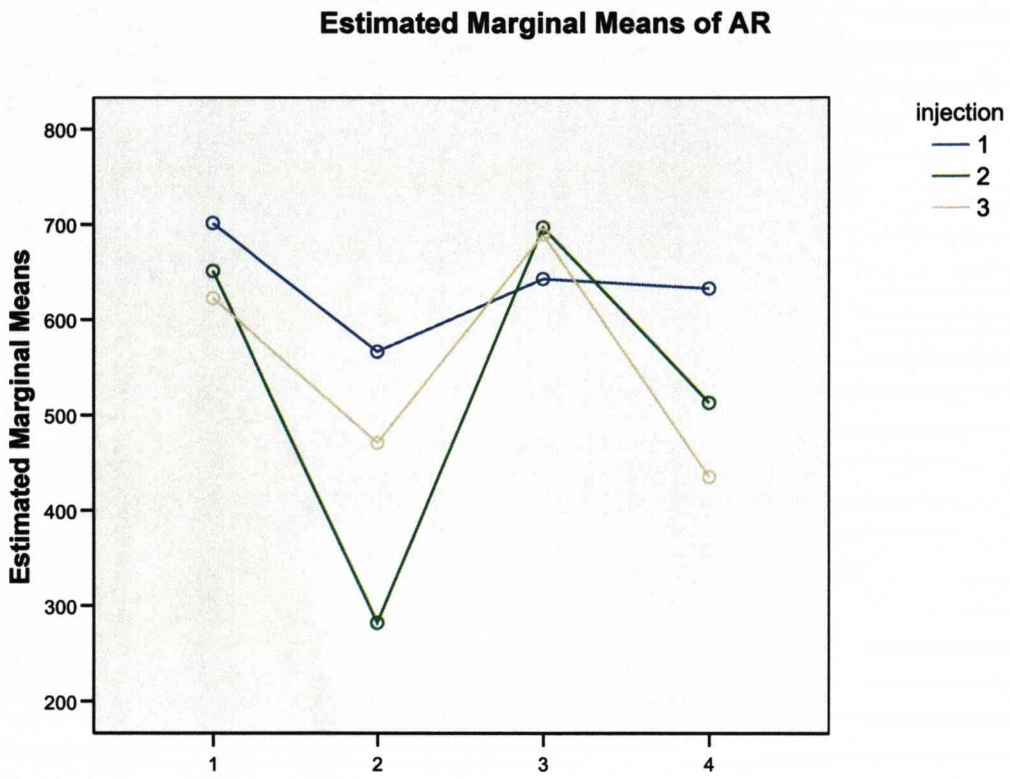
2. time

3. injection * time

Measure: AR

injection	time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	702.120	90.097	508.881	895.359
	2	566.973	86.830	380.741	753.206
	3	643.267	72.701	487.338	799.196
	4	633.153	66.421	490.695	775.612
2	1	651.627	87.264	464.464	838.789
	2	282.380	65.324	142.273	422.487
	3	697.220	70.368	546.296	848.144
	4	513.013	92.583	314.442	711.585
3	1	623.000	87.910	434.451	811.549
	2	470.967	72.429	315.623	626.311
	3	689.647	85.592	506.071	873.222
	4	435.080	81.962	259.289	610.871

Profile Plots



Appendix C

Short form McGill Pain Questionnaire

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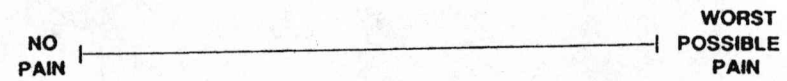
SHORT-FORM McGILL PAIN QUESTIONNAIRE

RONALD MELZACK

PATIENT'S NAME: _____ DATE: _____

	NONE	MILD	MODERATE	SEVERE
THROBBING	0) _____	1) _____	2) _____	3) _____
SHOOTING	0) _____	1) _____	2) _____	3) _____
STABBING	0) _____	1) _____	2) _____	3) _____
SHARP	0) _____	1) _____	2) _____	3) _____
CRAMPING	0) _____	1) _____	2) _____	3) _____
GNAWING	0) _____	1) _____	2) _____	3) _____
HOT-BURNING	0) _____	1) _____	2) _____	3) _____
ACHING	0) _____	1) _____	2) _____	3) _____
HEAVY	0) _____	1) _____	2) _____	3) _____
TENDER	0) _____	1) _____	2) _____	3) _____
SPLITTING	0) _____	1) _____	2) _____	3) _____
TIRING-EXHAUSTING	0) _____	1) _____	2) _____	3) _____
SICKENING	0) _____	1) _____	2) _____	3) _____
FEARFUL	0) _____	1) _____	2) _____	3) _____
PUNISHING-CRUEL	0) _____	1) _____	2) _____	3) _____



P P I

0	NO PAIN	_____
1	MILD	_____
2	DISCOMFORTING	_____
3	DISTRESSING	_____
4	HORRIBLE	_____
5	EXCRUCIATING	_____

Fig. 1. The short-form McGill Pain Questionnaire (SF-MPQ). Descriptors 1–11 represent the sensory dimension of pain experience and 12–15 represent the affective dimension. Each descriptor is ranked on an intensity scale of 0 = none, 1 = mild, 2 = moderate, 3 = severe. The Present Pain Intensity (PPI) of the standard long-form McGill Pain Questionnaire (LF-MPQ) and the visual analogue (VAS) are also included to provide overall intensity scores.

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